Level 8 Cell Culture Processing (BIO08045)

Lecture – "Bioreactor Modes of Operation – Part I"

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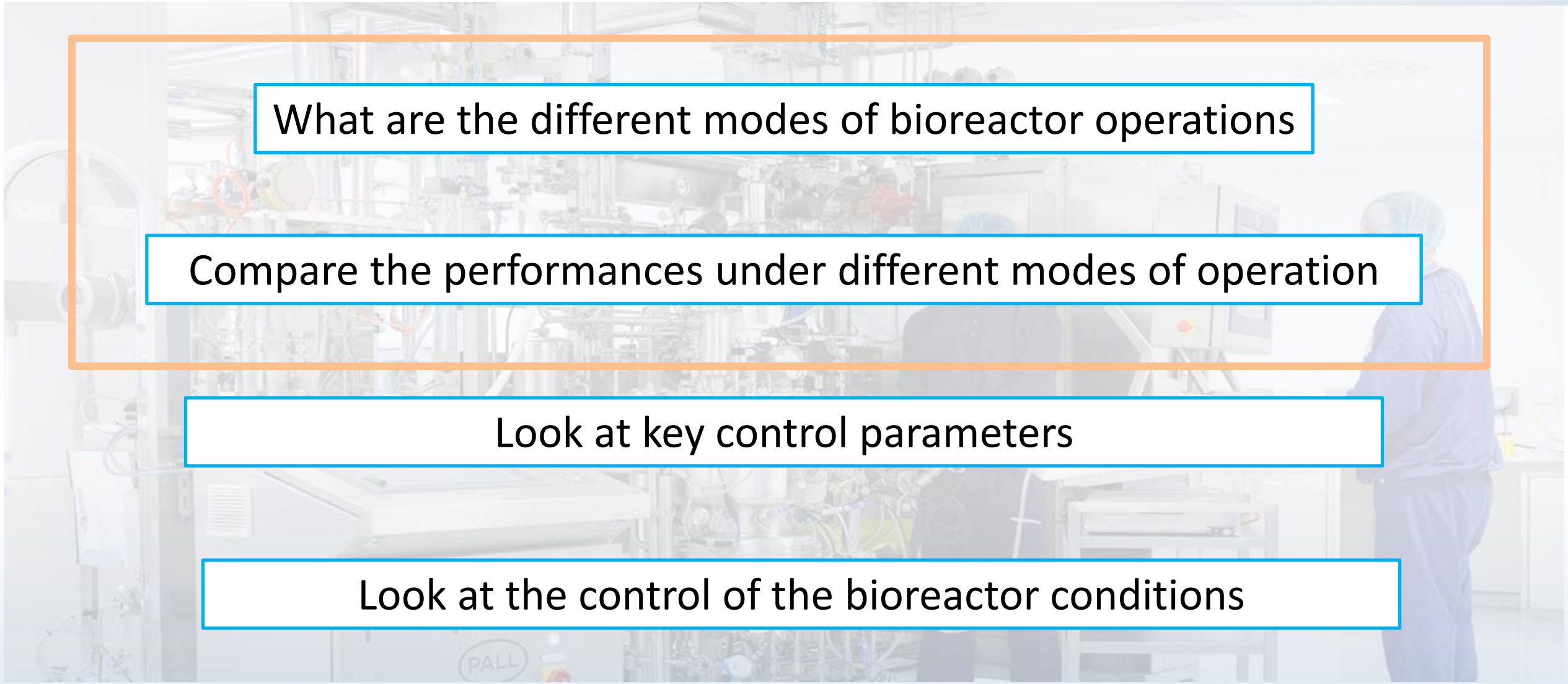
Learning Objectives



What are the different modes of bioreactor operations

Compare the performances under different modes of operation

Look at key control parameters

Look at the control of the bioreactor conditions

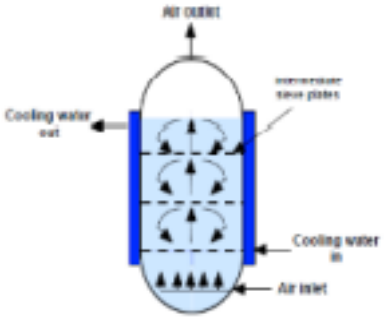
Reading Material

- *"Recent advances in large-scale production of monoclonal antibodies and related proteins"*. Shukla, A.A. and Thommes, J. 2010 Trends in Biotechnology Vol.28 No.5 p. 253-261
- *"Fed-Batch Cell Culture Process Optimization: A Rationally Integrated Approach"*. Jiang, Z. et al. BioProcess International 10(3) March 2012 p. 40-45
- *"Fed-Batch Mammalian Cell Culture in Bioproduction"*. Whitford, W.G. BioProcess International April 2006 p.30-40

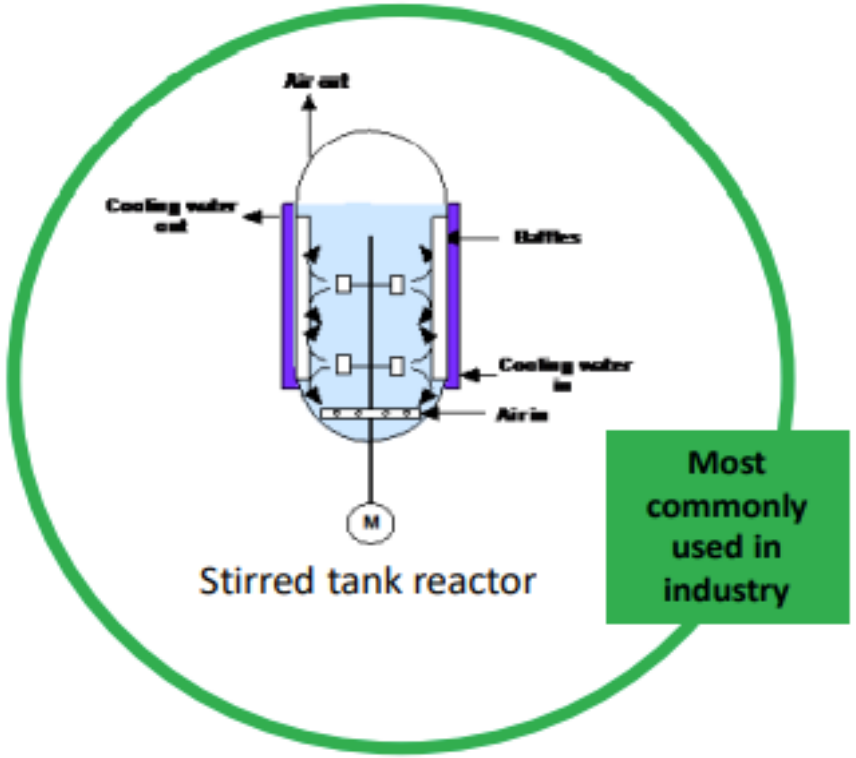
Lecture Topics

- Modes of Bioreactor Operations
- Continuous / Perfusion Culture System

Types of Bioreactors

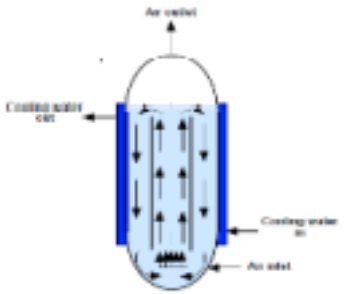


Bubble Column reactor



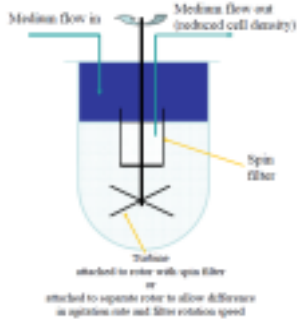
Stirred tank reactor

Most commonly used in industry

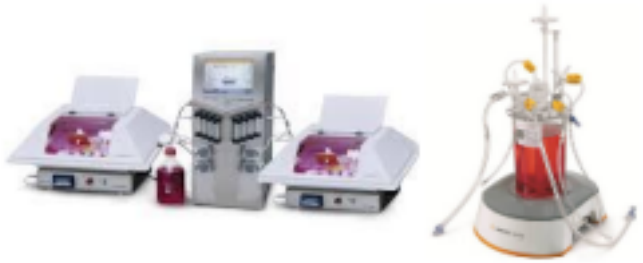


Airlift Bioreactor

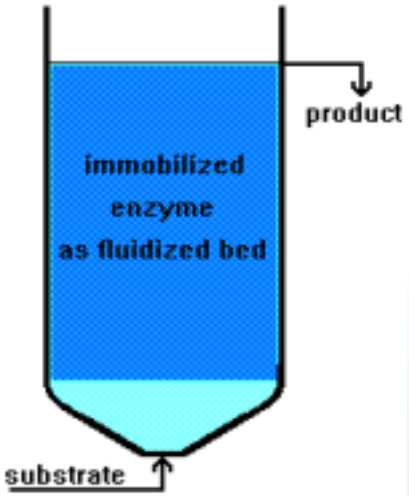
Internal spin filter



Filtration-cell recycle bioreactors

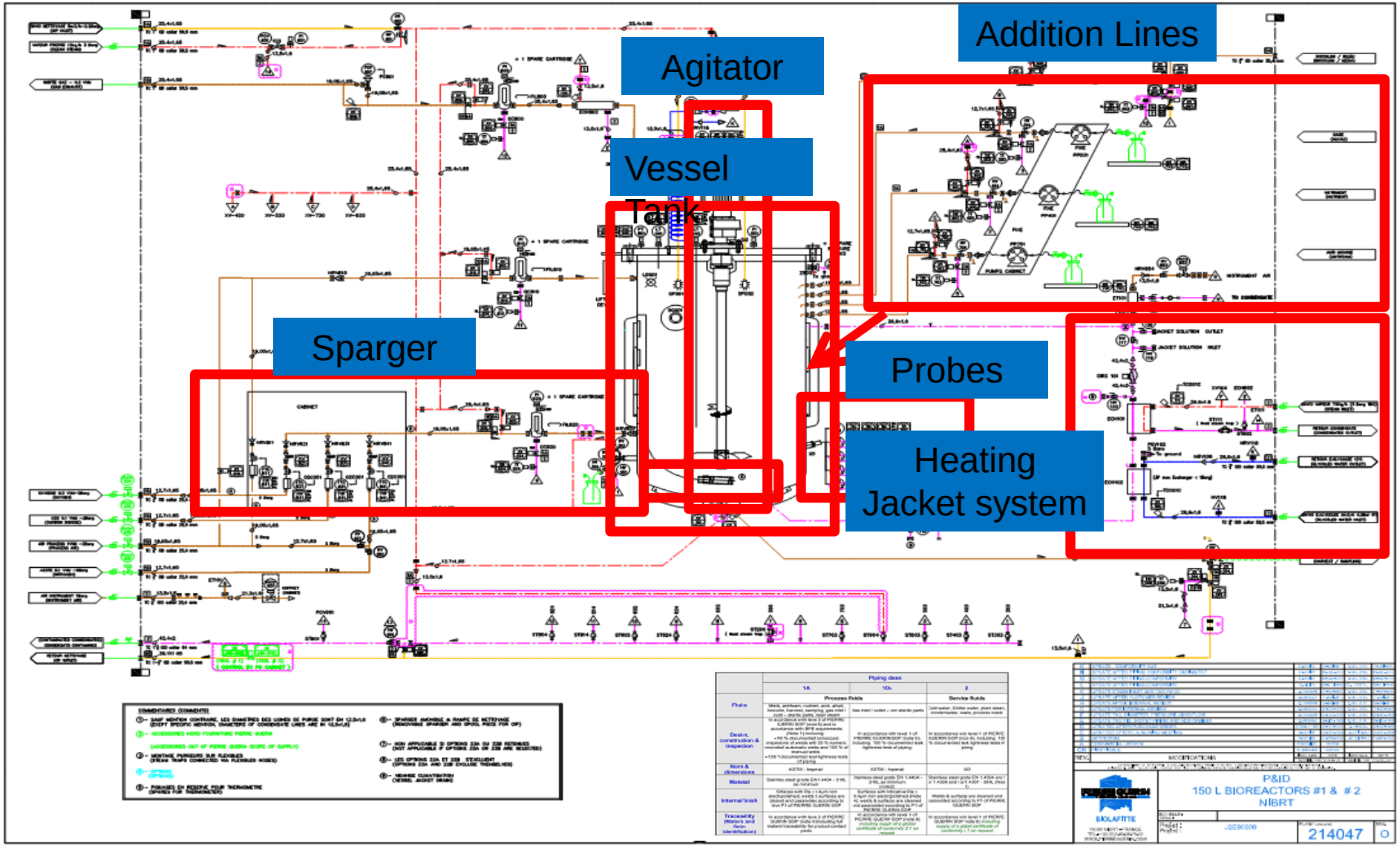


Disposable bioreactors



Fluidised bed bioreactor

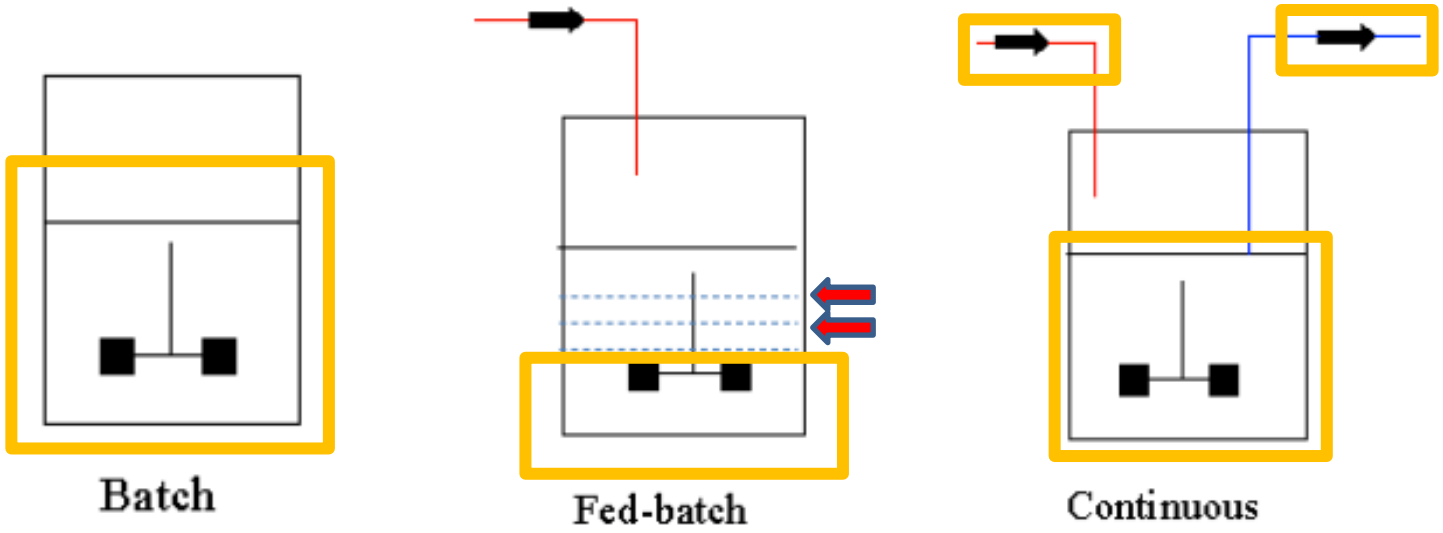
Reactor Components



Three Common Modes of operation

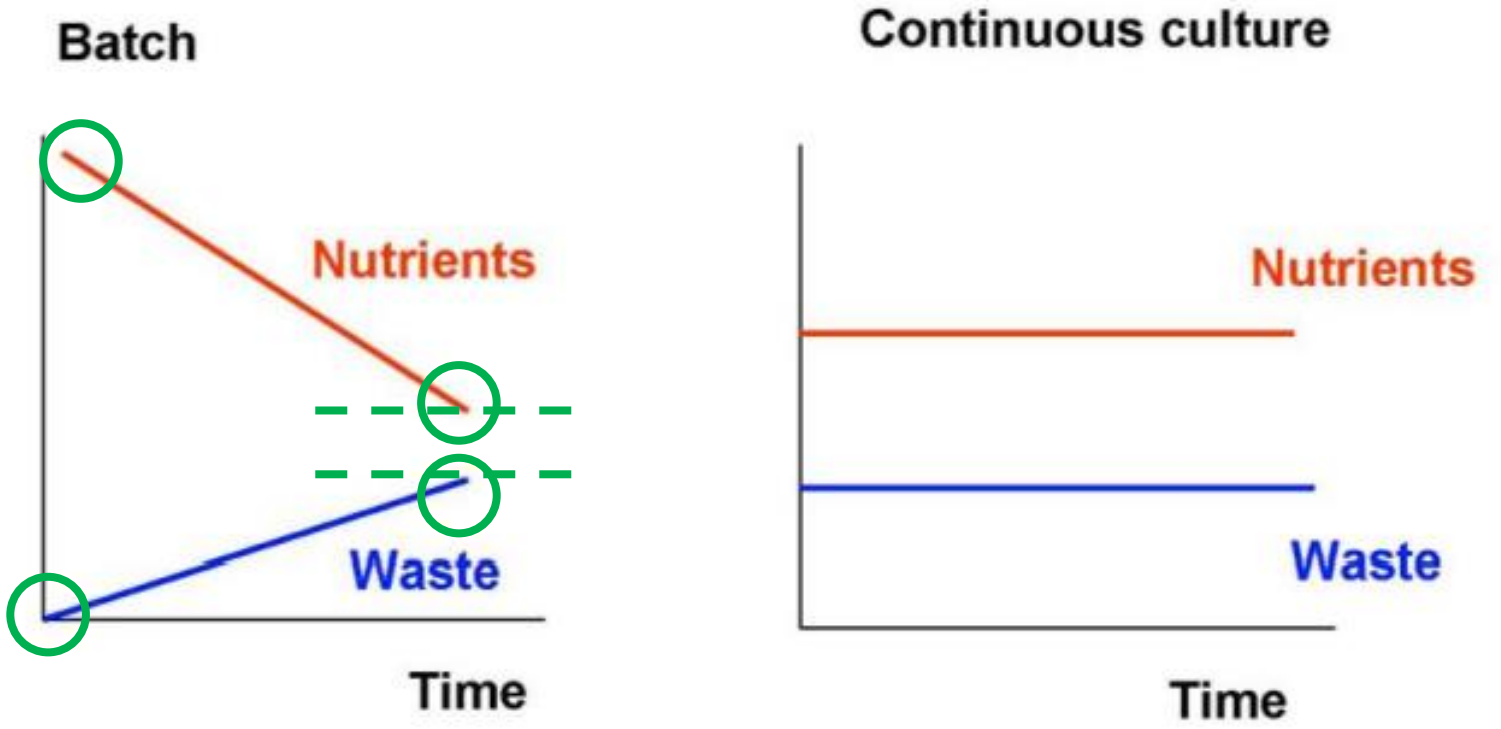
1. Batch: cells inoculated into fixed volume, consume nutrients and metabolites accumulate
   - Cells division stop when all nutrients are used up - environment is constantly changing.
2. Fed Batch: can prolong life of batch culture by feeding with fresh media
   - Start with small volume and increase until vessel is full
   - Usually feed in substrate (glucose)
3. Continuous flow-culture: withdrawal of medium and cells and addition of new medium at constant rate (Biostat)
   - No fluctuations in nutrients, metabolites or cell number

Mode of bioreactor operation



1. Batch reactors are the second most commonly used.
2. Fed batch bioreactors are most commonly used to produce biological products using mammalian cell expression systems.
3. Continuous reactors are not used often for large scale production of biochemicals but are finding increasing acceptance

Batch Design vs. Continuous Culture

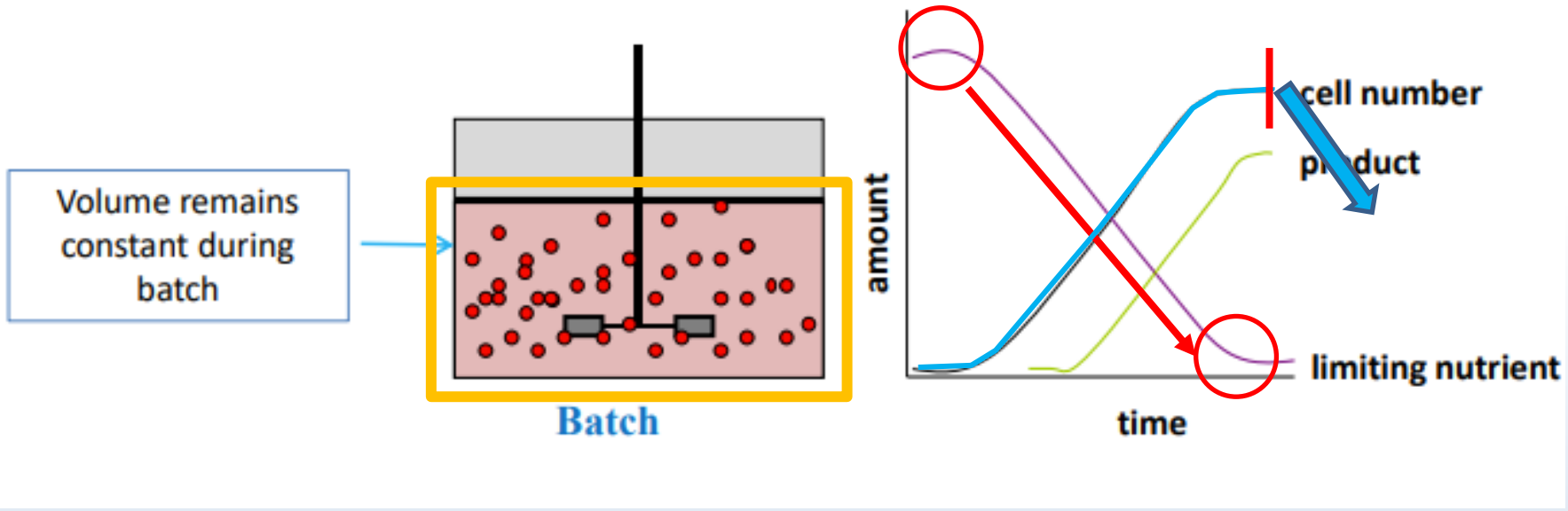


Continuous culture: nutrients and fermentation by-products stay at the same concentration. Continuous culture mimics the blood stream in the body

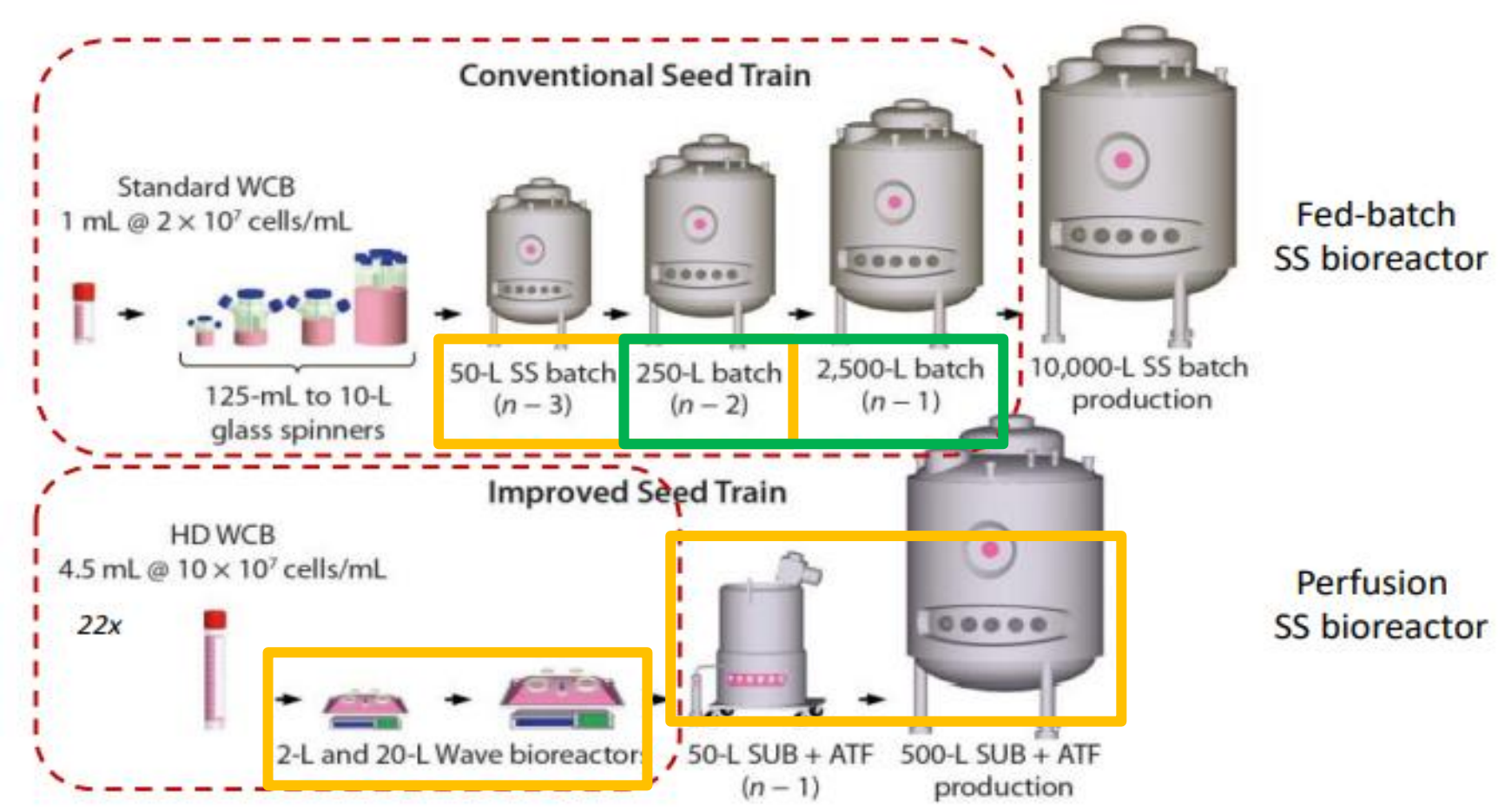
Batch Culture

Batch Culture

1. Media added once prior to start of culture.
2. Cells added: cells are inoculated into fixed volume – they consume nutrients and metabolites accumulate.
3. Cells division stops when all nutrients are consumed, and toxic by-products build up



Bioreactor Seed Train



Adapted from: Wright, B. et al (2015) A Novel Seed-Train Process : Using High-Density Cell Banking, a Disposable

Bioreactor, and Perfusion Technologies BioProcess International 13(3)s

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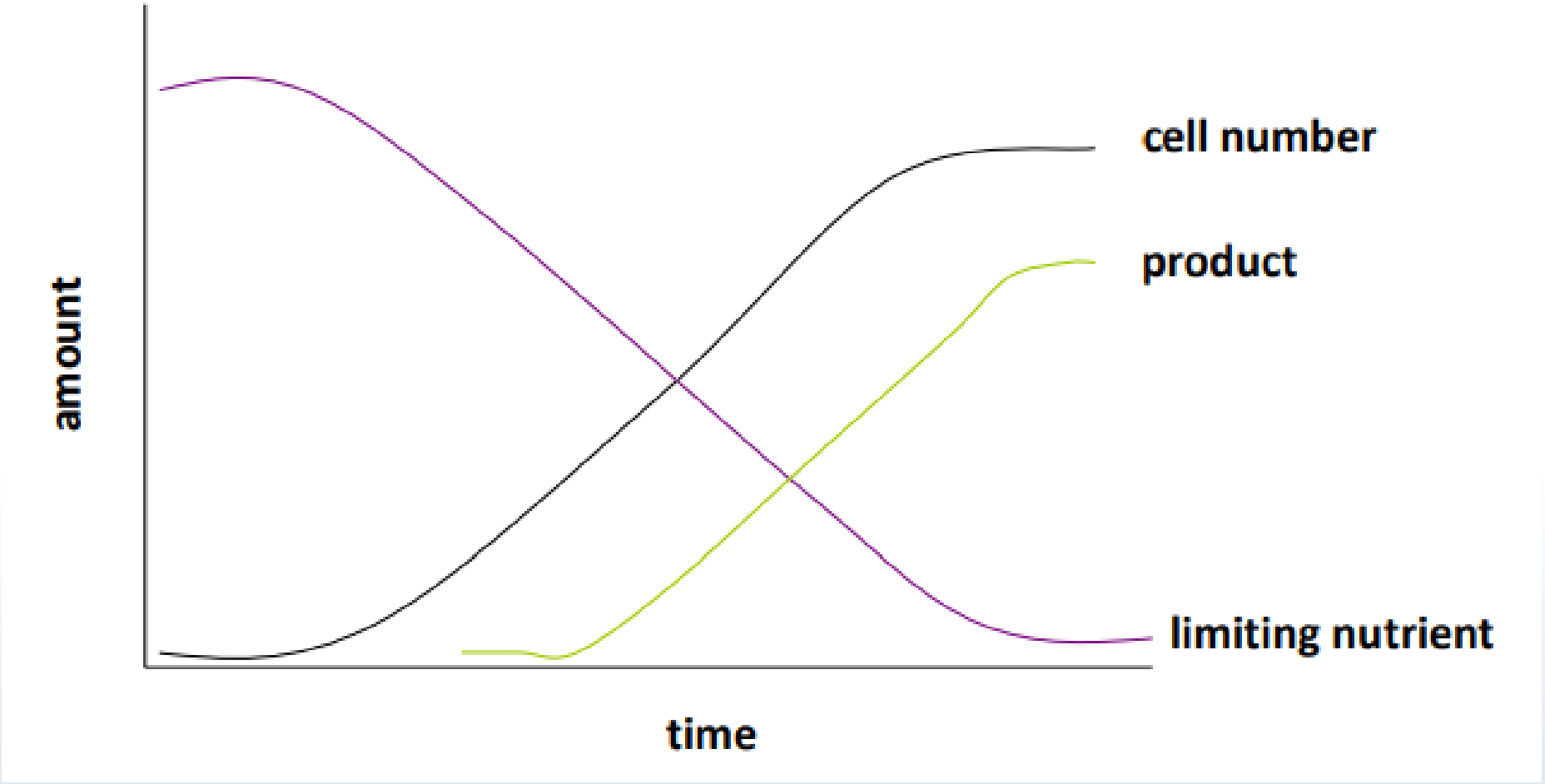
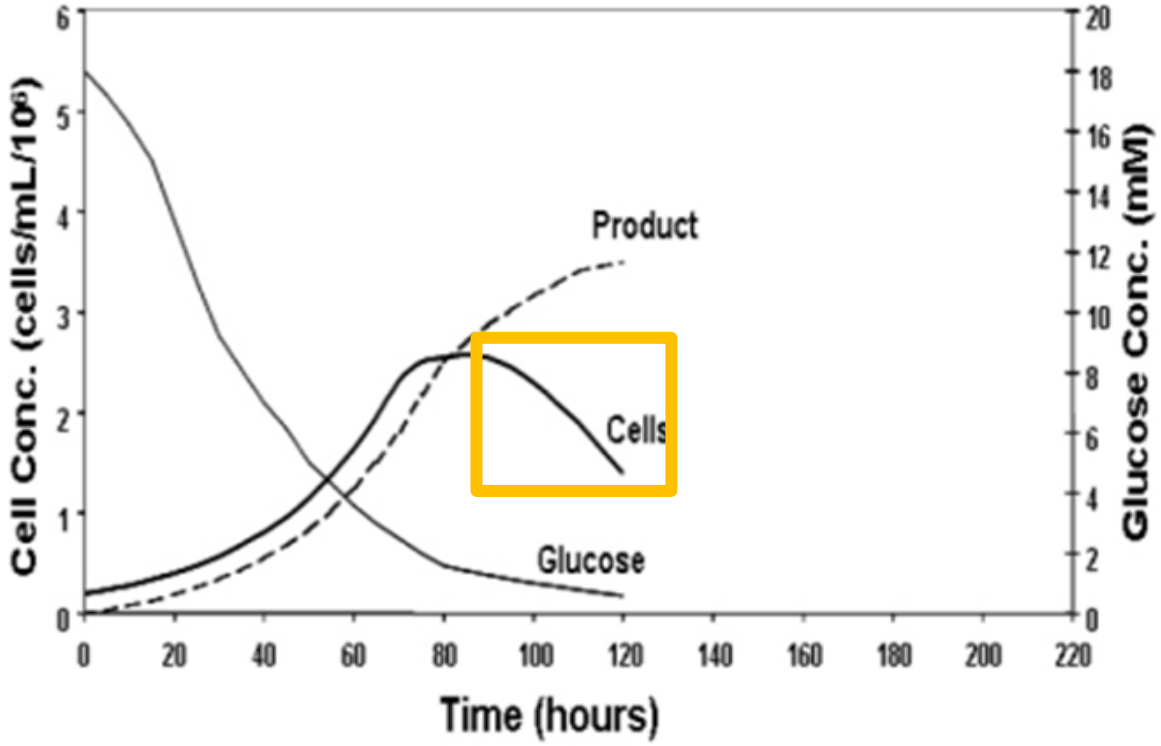
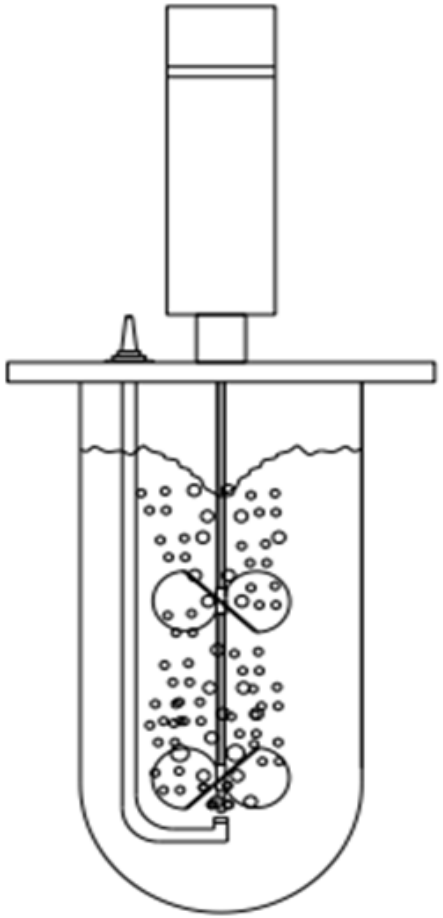
Batch Culture – Closed System



Batching Types

- Single Batch
  - 7-14 days
  - Run is over when there are no more nutrients





Advantages/Disadvantages of Batch Culture

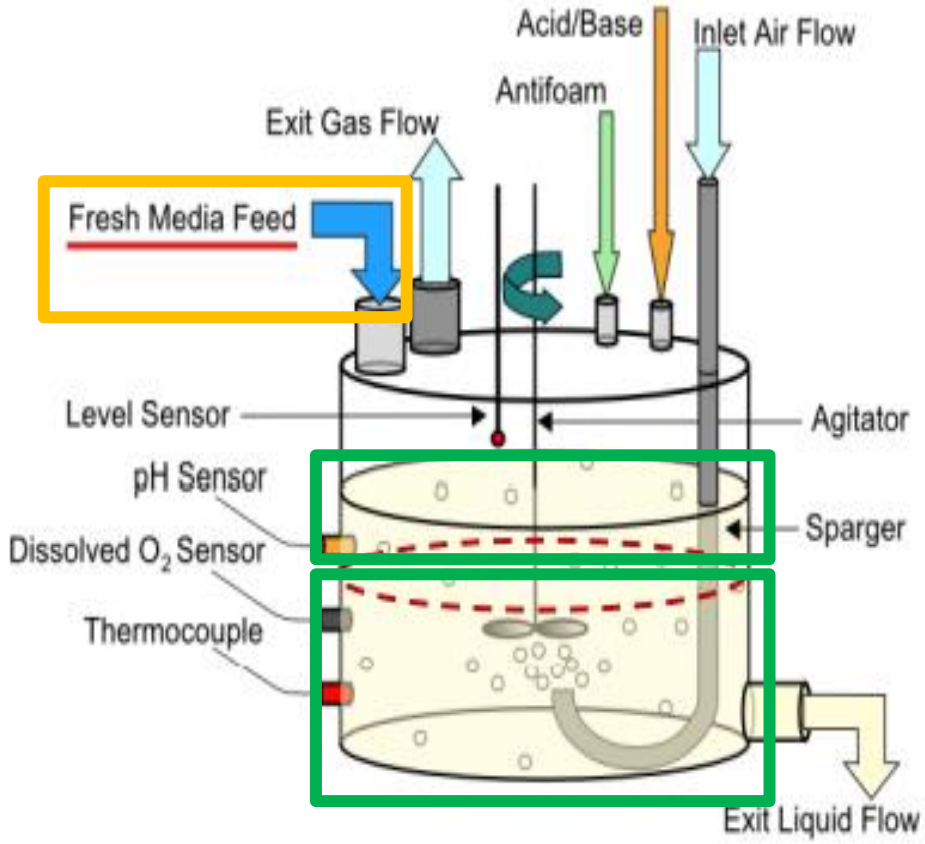
- Advantages
  a) Well characterised, most common method.
  b) Ease of operation and maintenance.
  c) Low installation costs.
  d) Reduced risk of contamination.
  e) Easier to validate.

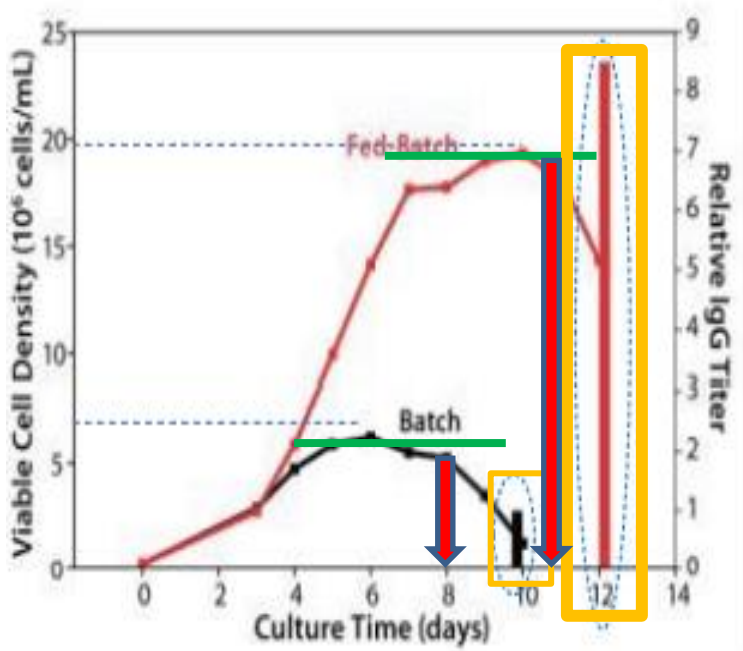
- Disadvantages
  a) Lower productivity
  b) Risk of substrate reducing growth/productivity.
  c) Lower maximum cell densities.
  d) Accumulation of toxic by-products over time.
  e) Product is in contact with proteolytic enzymes at 37°C throughout run.

Fed-Batch Operating Mode

- Inoculate reactors at 75 – 90% of final working volume
- Fed up to the final working volume during the course of production
- Terminate culture based on a pre-defined criterion e.g. % of viable cells, specific nutrient concentration etc.
- Duration: varies from circa 8 to 21 days
- Typically follow by harvest using centrifugation and/or depth filtration

Fed-Batch System





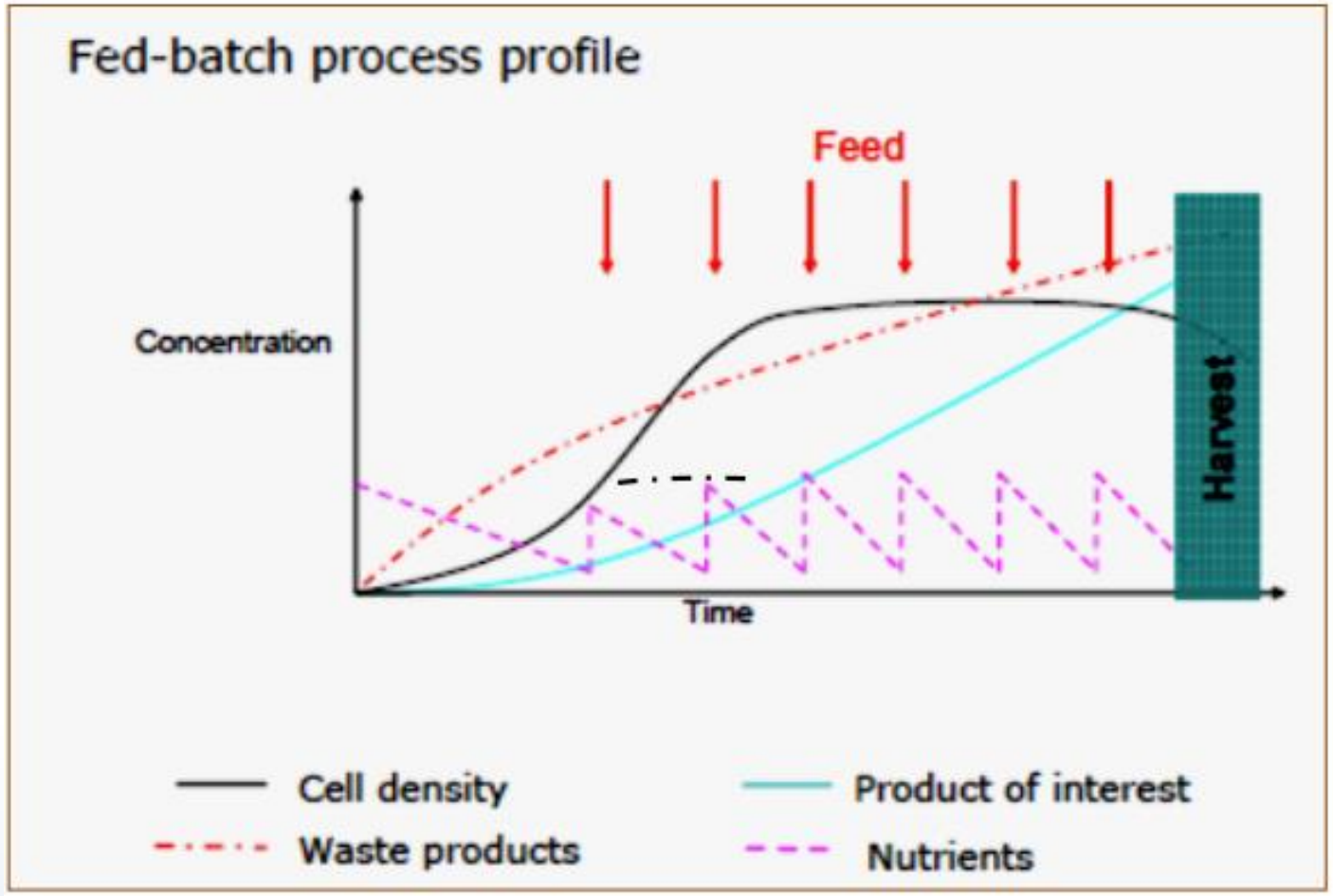
From:

http://www.ecs.umass.edu/che/henson_group/research/bioreactor/fermenter.png

Adapted from: Jiang, Z. et al. (2012) Fed-Batch Cell Culture Process Optimization BioProcess International V13 March 2012

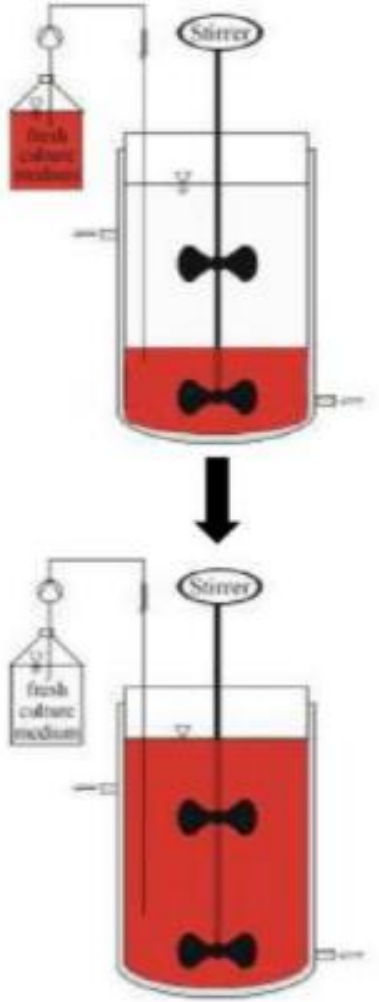
http://www.bioprocessintl.com/upstreamprocessing/cell-culture-media/fed-batch-cellculture-process-optimization-328055/

Fed-Batch Production Profile



From CMC Biologics online resource: Perfusion- - Jeopardy or the Ultimate Advantage (2009) by Carstens, J.N., Clarke, H.R.G., and Jensen, J.P. http://www.cmcbio.com/Portals/0/CMC/docs/perfusion.pdf

Fed-Batch Bioreactor Design



Advantages:

- easy to perform
- simple upscaling possible
- prolonged cell growth and production (compared to batch mode)

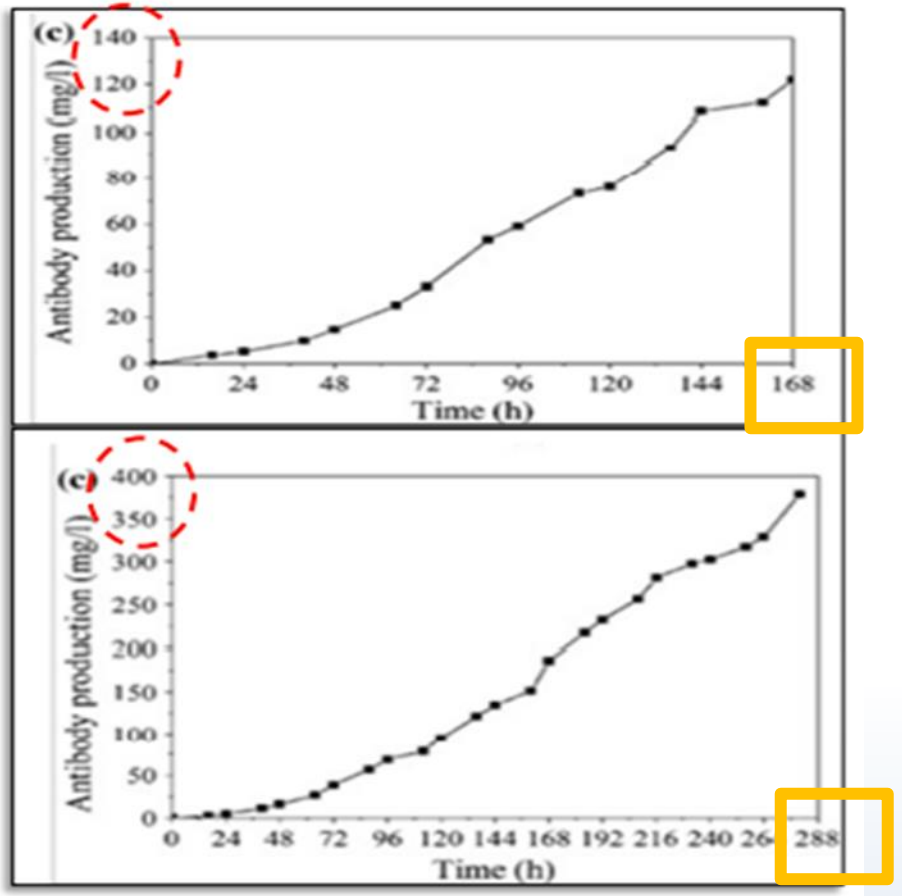
Disadvantages:

- low cell densities
- low productivity
- often product degradation

Batch –V- Fed-Batch Production

- A serum free medium and feeding strategy was developed for a fed-batch process by rational design and statistical methods
- Optimized mixture of DMEM:F12:RPMI1640 (2:1:1)
- A titre of 90.95mg/L was achieved, which out-performed commercial SFM EX-CELL ™ by 18%
- Fed-batch strategy utilized glucose measurements to yield a **final Mab titre of 378ml/L - a three-fold increase on batch studies**

Zhang H., et al, 2013



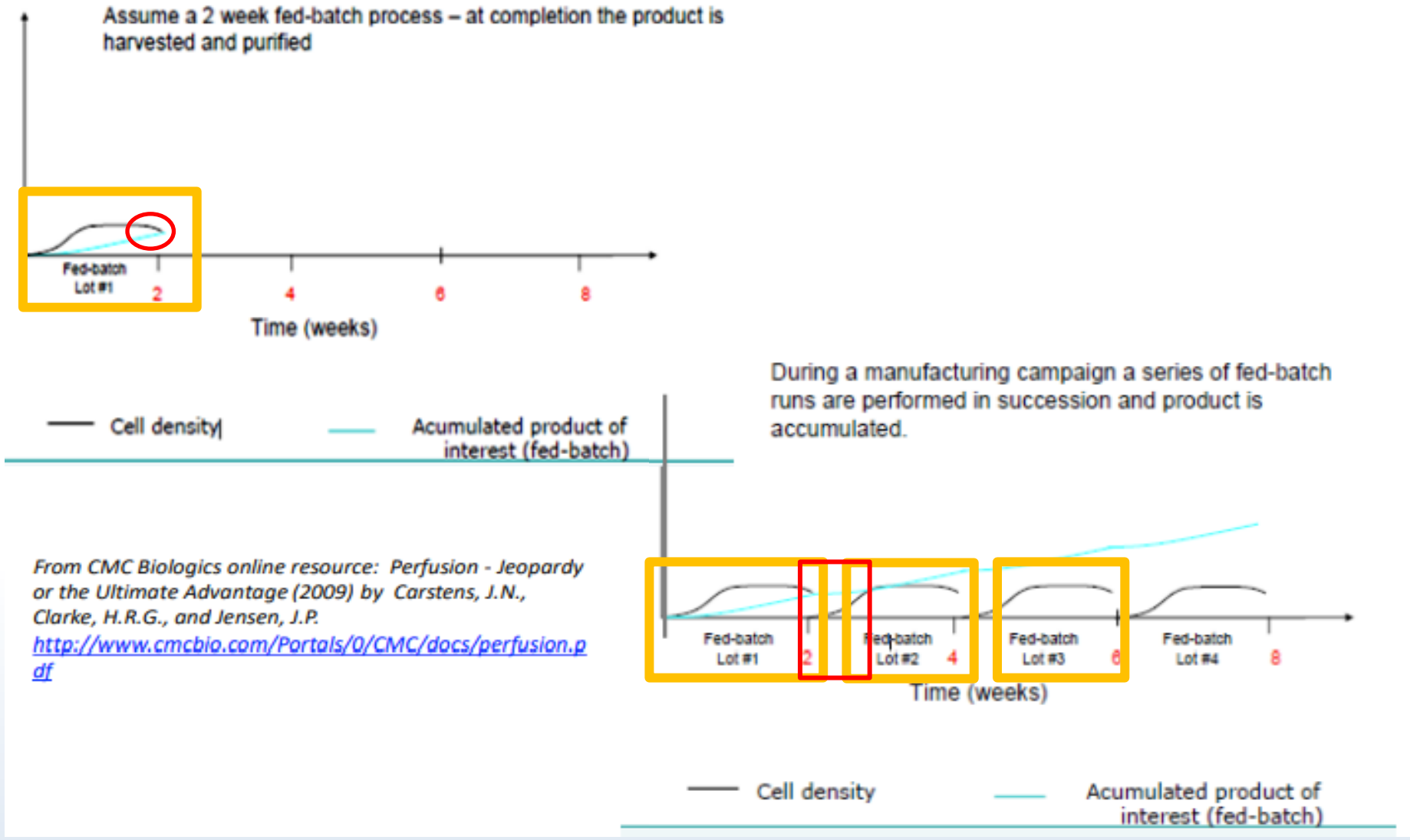
Differences in Mab production in the batch culture (top) and fed-batch culture process (bottom)

Advantages/Disadvantages of Fed-Batch Culture

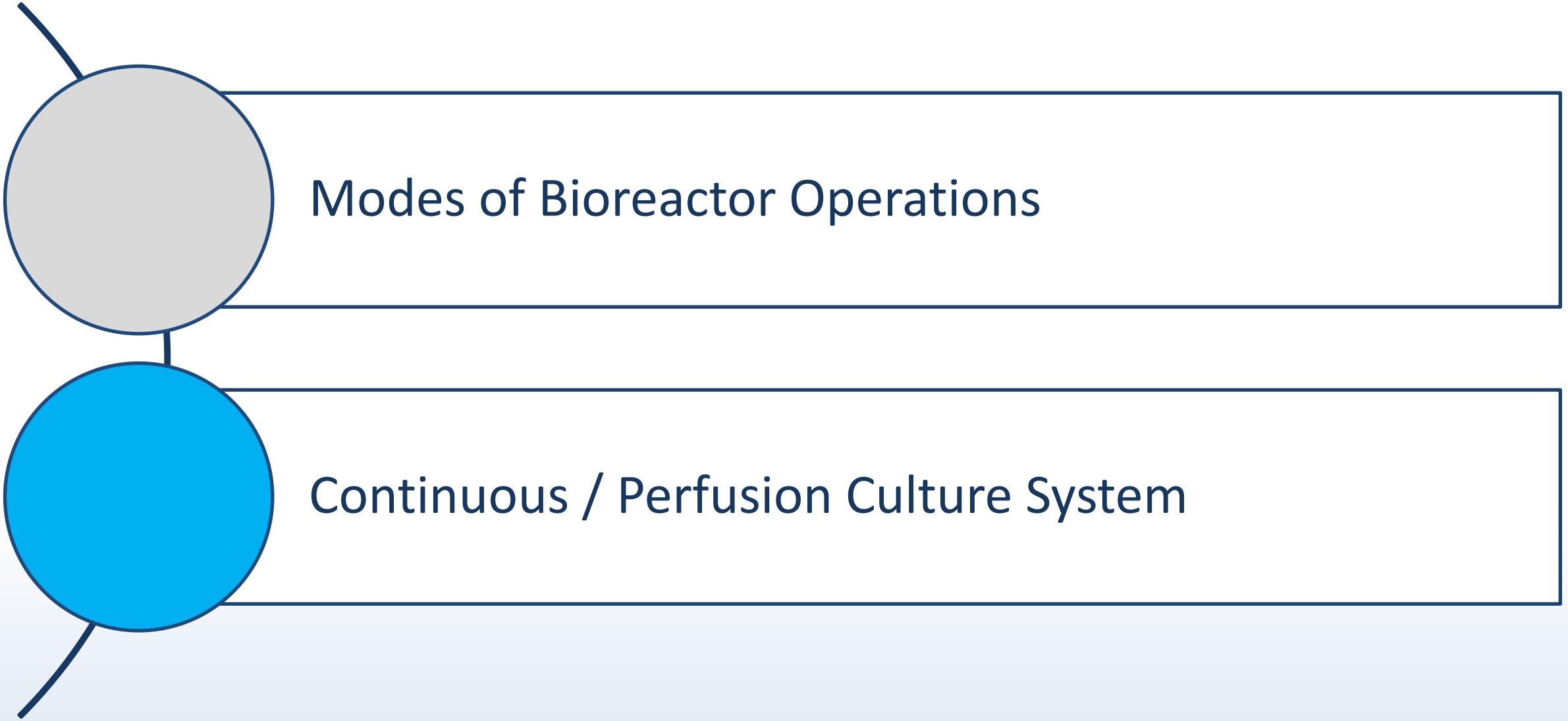
- Advantages
  a) Growth rate and production rate extended
  b) Increased control of cell culture environment
  c) Growth rate, or production rate, extended while maintaining residual substrate concentration at a relatively low level.
  d) Higher product concentrations (titres) than continuous culture – benefits in downstream processing
  e) No cell retention devices required
  f) May be operated as repeated fed-batch to further improve productivity

- Disadvantages
  a) Requires large bioreactor designs (>10,000L) for high demand products– higher capital costs
  b) Large-scale bioreactors can create more complex scale-up challenges
  c) Different age profiles for the protein product produced over an extended run
  d) Requires sophisticated biosensors and computer automation to control feed rate and maximize overall productivity.
  e) Increased medium volume required
  f) Complex feed control strategy required

Fed-Batch Campaigns



Lecture Topics



Continuous or Perfusion System

- **Continuous or perfusion system (open),** with continuous feed of medium and removal of ‘spent’ medium : 2 major types
  - **Chemostat** culture where the cells and media are removed continuously from the bioreactor so growth rate remains constant
  - The state of the culture is dependent upon the flow rate of fresh medium
  - Seeks to maintain a constant chemical condition with respect to nutrients
  - **Biostat** - maintain a constant cell concentration

Continuous System cont/d.

- **Perfusion** culture where the cells are retained in the bioreactor and only media and waste products are removed are becomingly increasingly popular in production as much higher cell no's can be achieved
  - **Maximum cell number in batch or fed-batch is ~ 1 or 2 x 10^7cells/ml while you can obtain up to 10^8 cells/mL in continuous**

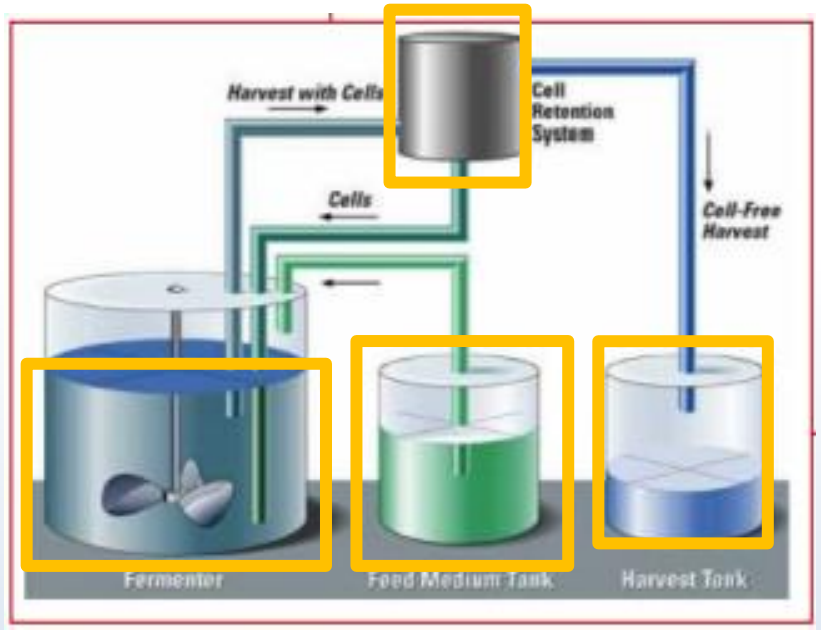
Perfusion – Historical Perspective

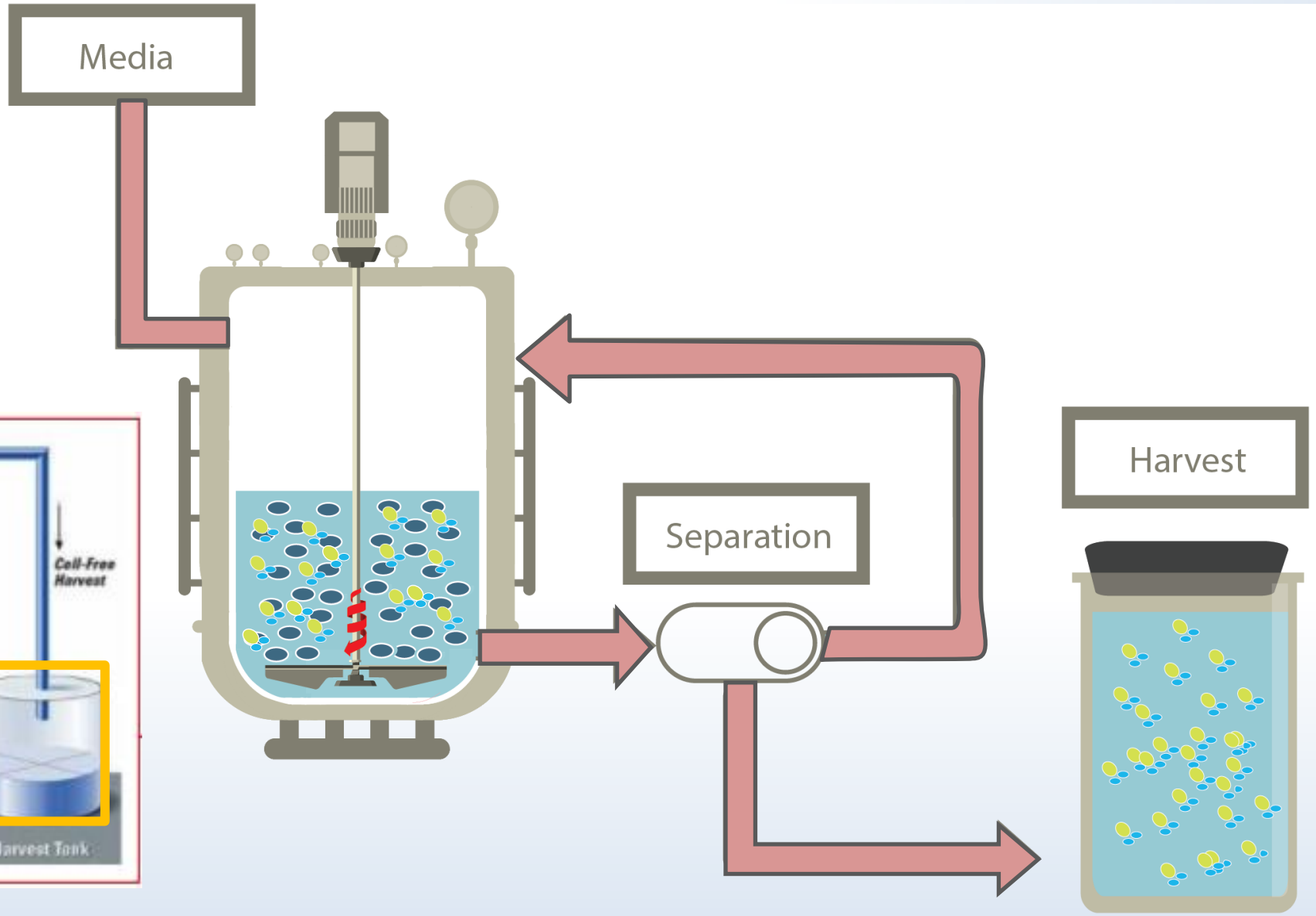
- Perfusion systems for mammalian cell culture were developed in the 1990's
  - Product titres were low due to low expression rates
  - Cell culture media were relatively simple – basal media with serum predominated
- During the 90's, industry moved away from perfusion as media improvements and improved expression systems led to improvements in fed-batch production
  - Fed-batch was simple and reliable compared to perfusion which was seen as complex and prone to high failure rates

Perfusion today

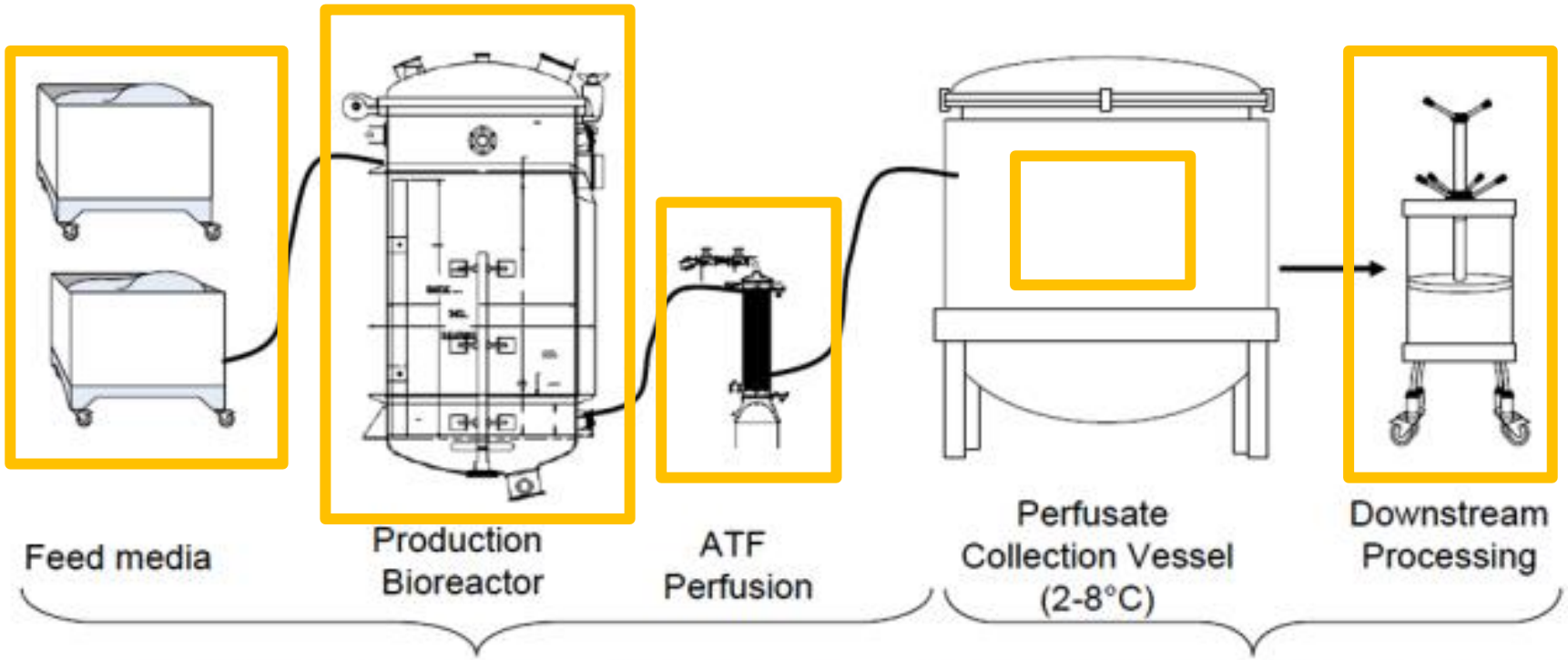
- Increasing rates of adoption - cell culture equipment and controls are now more sophisticated
  - Improved understanding of perfusion techniques
  - Improvements in sterile filtration
  - More sophisticated pumps with feed back control loops
  - Media improvement
- Key driver favouring perfusion – reducing cost of goods and capital investment
  - Increased price competitiveness required as products come off patent
  - Pressure to reduce costs of finished product to patients
  - Continuous manufacturing is more capital equipment effective

Continuous Culture





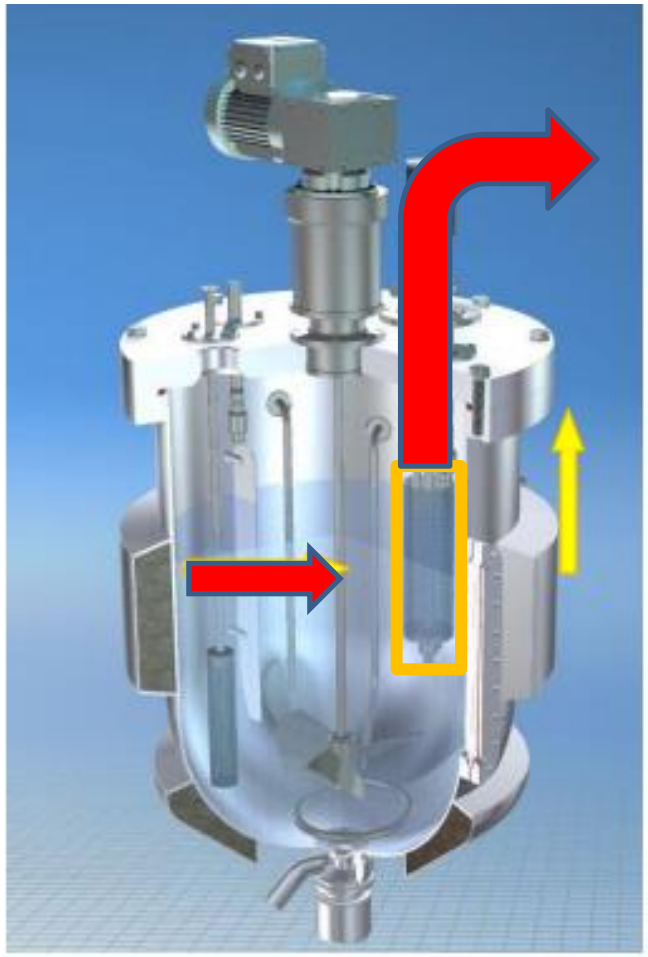
Manufacturing Logistics



Cell Retention Devices

- Many devices developed by companies to retain cells e.g.
  a) Acoustic Cell Filter : high frequency, low energy acoustic sound to aggregate cells on filter
  b) Spin filter : rotating mesh cage to allow medium flow but prevent cell wash-out
  c) ATF – alternating tangential flow device

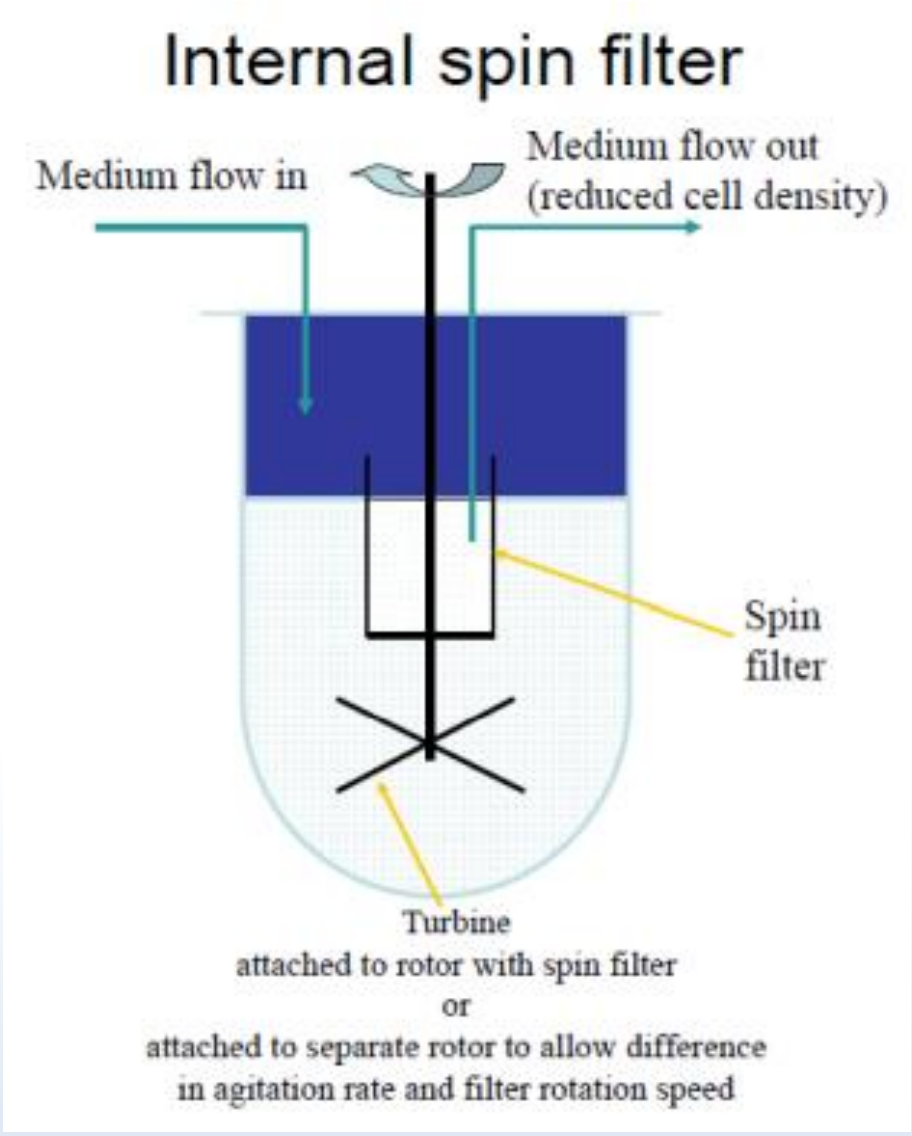
Spin Filter Design for Continuous Bioreactor



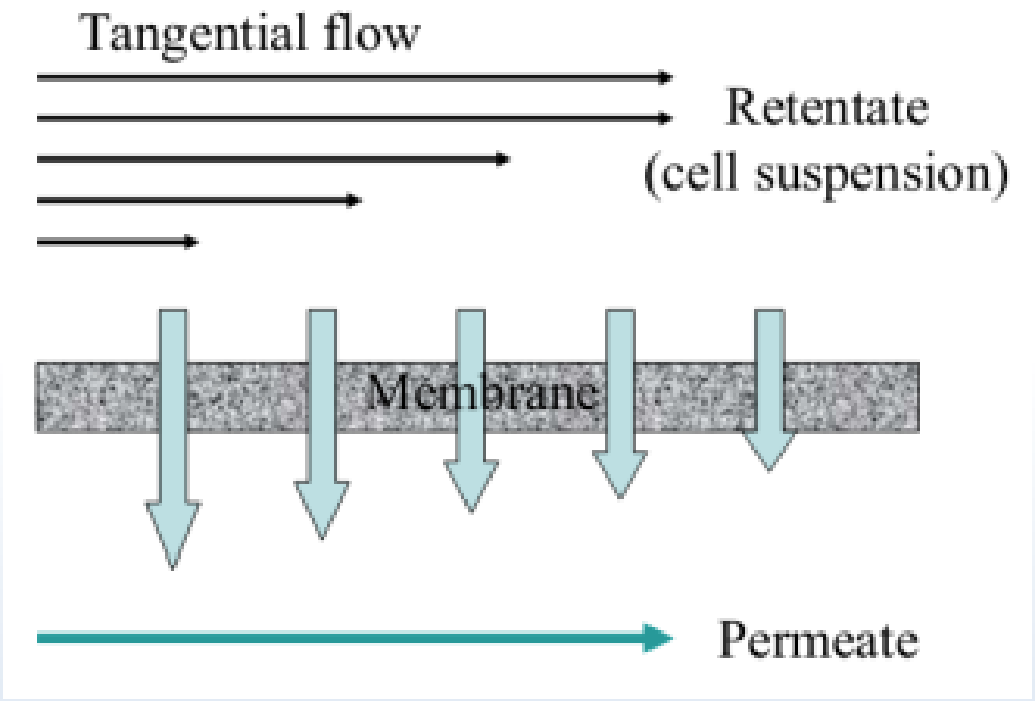
Spin Filter Design for Continuous Bioreactor

- Agitation systems may consist of an agitator and baffles
  - Agitator consists of drive motor, speed control, shaft and impeller
  - A control system comprising a revolution counter (RPM) and a power monitoring system to monitor the power levels drawn by the drive motor.
- The agitator may be either top or bottom mounted, direct or magnetic drive.
  - **Bottom mounted** agitators require a shorter shaft but require higher maintenance due to damage of seals in drive shaft
  - **Top mounted** agitators are not submersed in culture resulting in reduced risk of contamination.

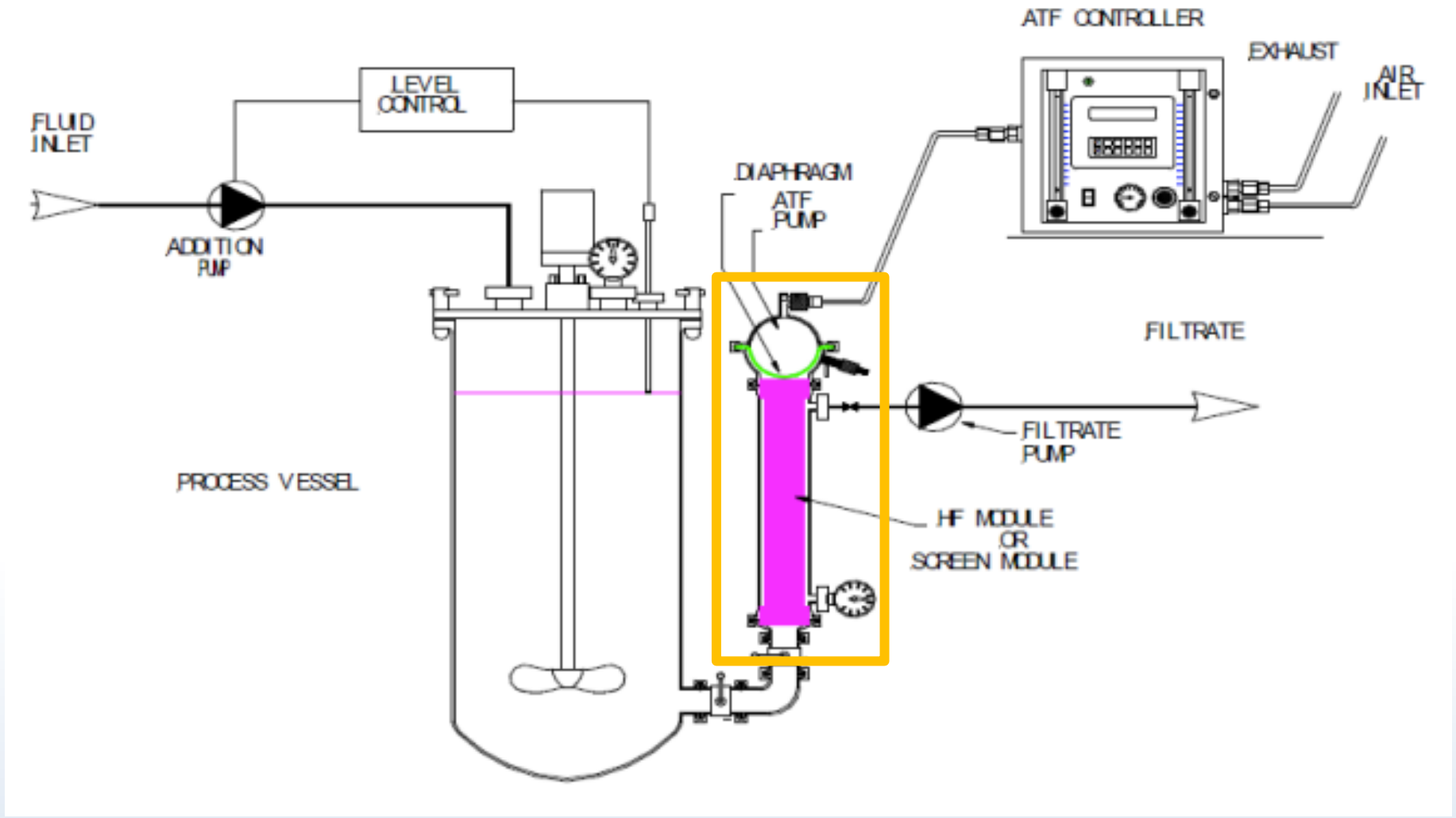
Spin Filter Design for Continuous Bioreactor



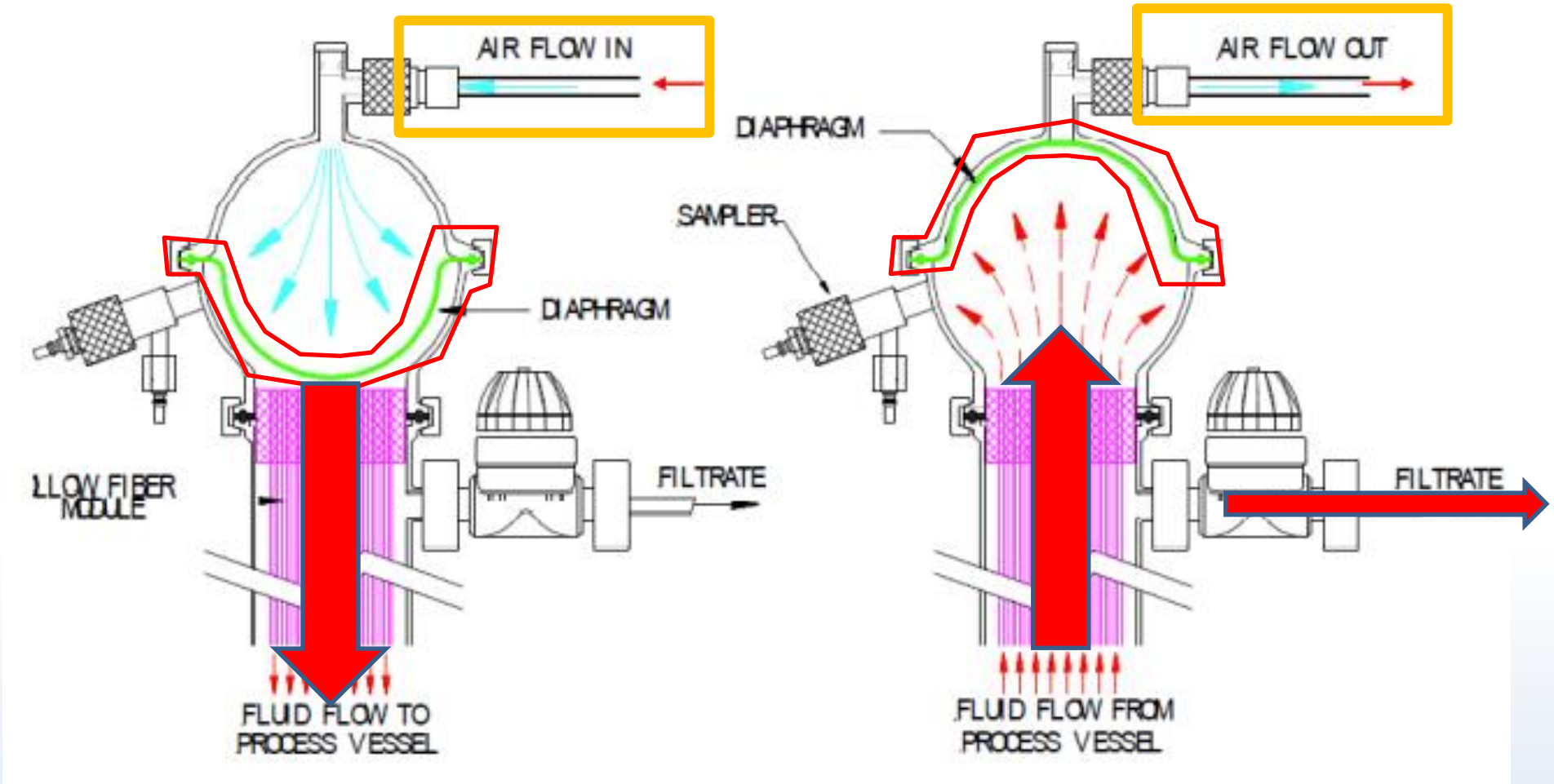
- Filtration including cross flow filtration (tangential), hollow fibre, spin filter.



ATF Cell retention Device



ATF Cell retention Device



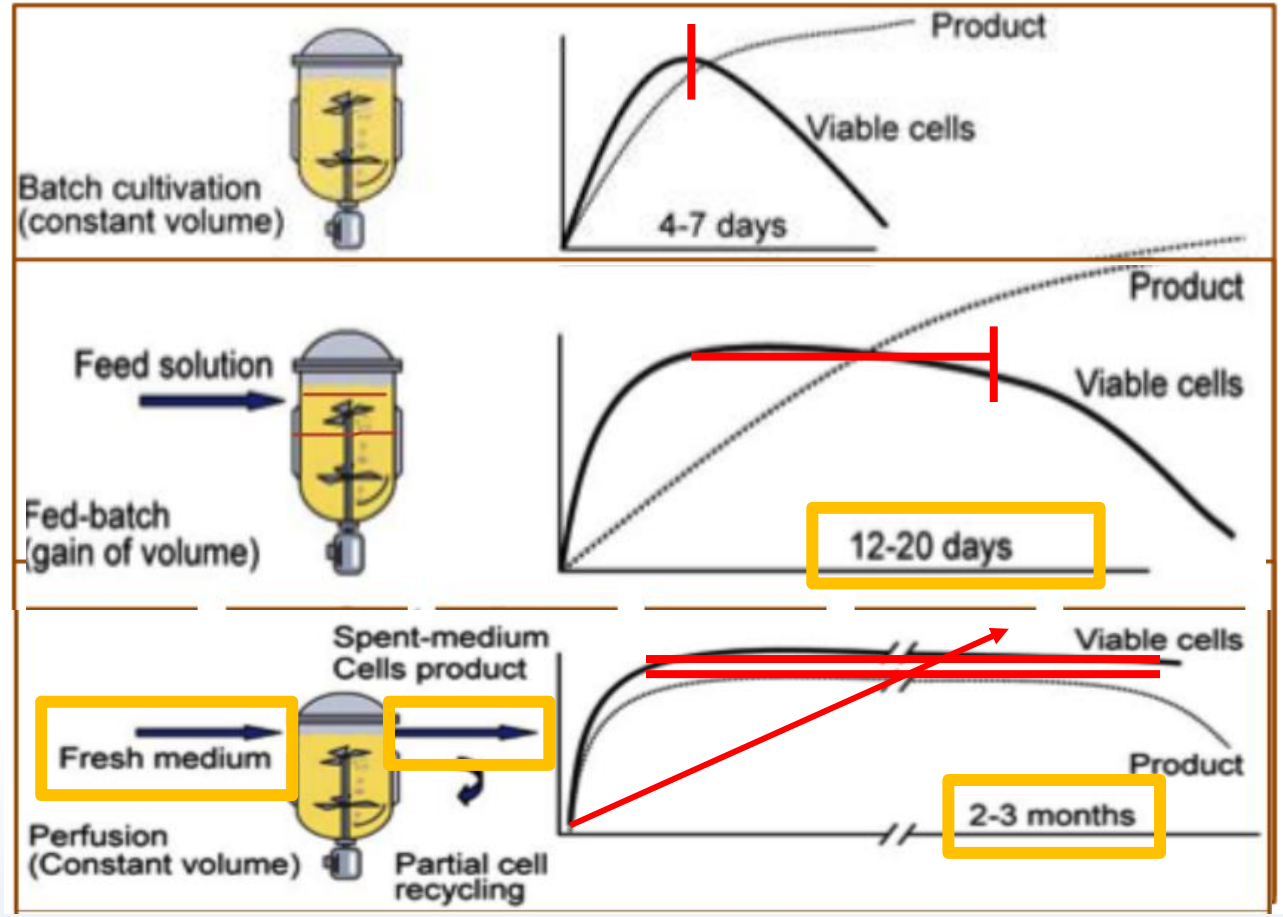
Chemostat / Perfusion System

- **Advantages**
  - Ideal for products where there are product quality concerns
  - Bioreactors are continuously harvested; labile producers can be processed quickly
  - Operating conditions of the bioreactor are constant (no limitation / inhibition) and can be precisely controlled – critical for some products
  - High volumetric productivity from bioreactors compared to fed-batch systems
  - Much higher peak cell densities are achievable
  - Bioreactors can operate continuously for expended periods at high cell density
- **Disadvantages**
  - Can be technically complex with a high technical demand

Fed-Batch –v- Perfusion

	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

Material Balances



Advantages of Perfusion Bioreactor vs. Fed-batch

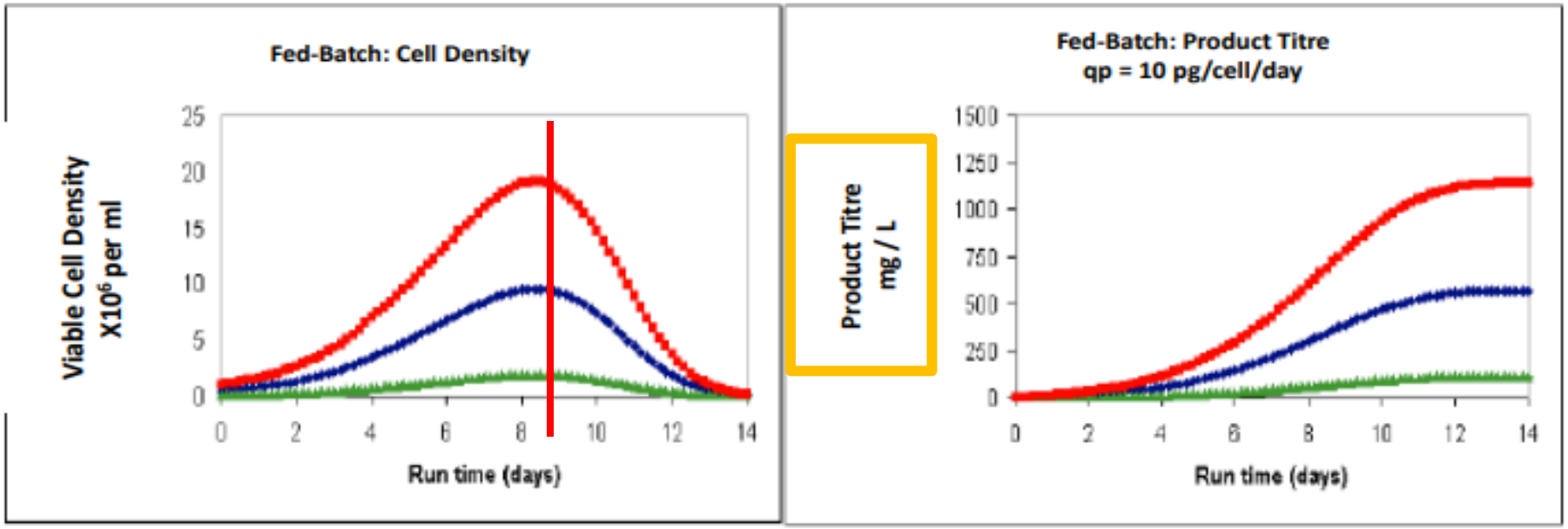
1. Continuous bioreactors are typically smaller in size than batch or fed-batch because of higher turnover rates (20 – 2500L).
2. Reasonably high cell density achievable and thus reasonably high volumetric productivity because of cell recycle systems.
3. Continuous bioreactors yield more uniform product – cells at same physiological state throughout – constant environmental conditions with constant growth rates and cell densities.
4. Constant yield, productivity and conversion rates due to continuous removal of product with no hold steps.
5. Shorter exposure time of product to potentially harsh production environment.

Disadvantages of Perfusion Bioreactor vs. Fed-batch

1. Higher usage of expensive cell culture media with less utilisation of the media nutrients. May be economically more expensive.
2. Need for a larger and continuous protein capture system with more waste materials to be disposed of.
3. Requires large media feed and storage systems for continuous substrate supply.
4. Requires expensive, high quality and reliable equipment with more complex control and monitoring systems.
5. Because of the extended period of continuous culture batch runs there is an increased risk of quality impact to the product due to the potential for genetic drift in the cells.

Productivity – Fed-Batch Model

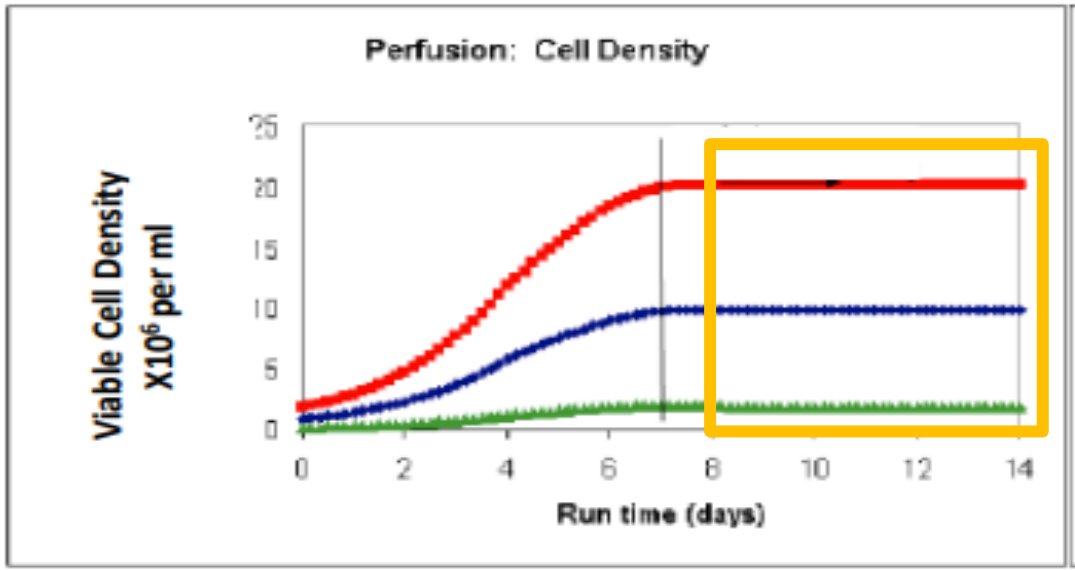
Using a 14 day fed-batch bioreactor with a constant specific productivity (qp)

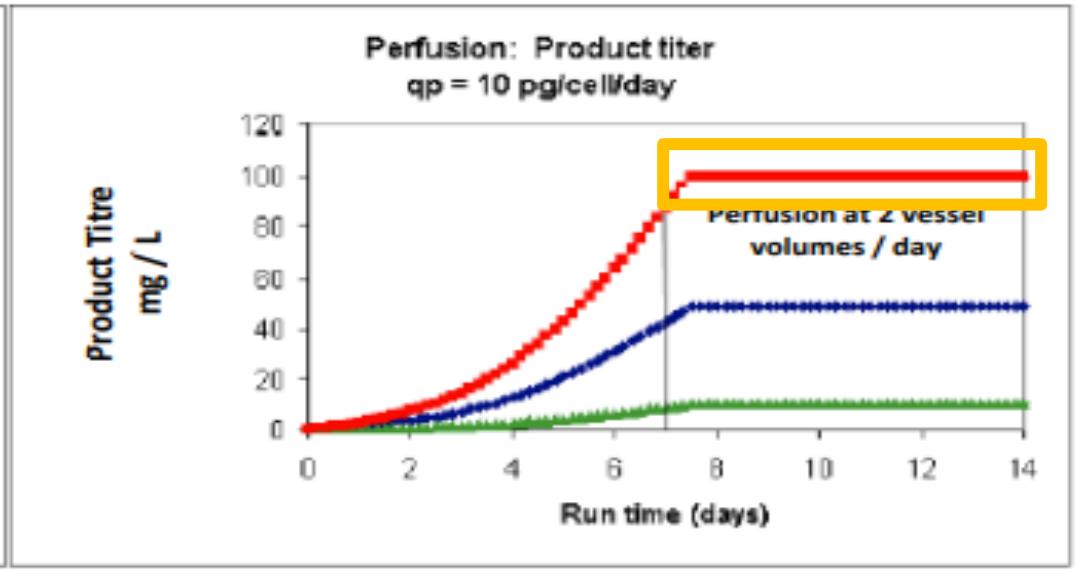


Peak day 8 cell density (million cells/ml)	volumetric productivity (mg/day/bioreactor liter) during a 14 day period	
	Fed-batch	Perfusion
20	81	
10	41	
2	8	

Productivity – Perfusion Model

Using a 14 day perfusion bioreactor with a constant specific productivity (qp) and harvesting 2 vessel volumes per day from day 7



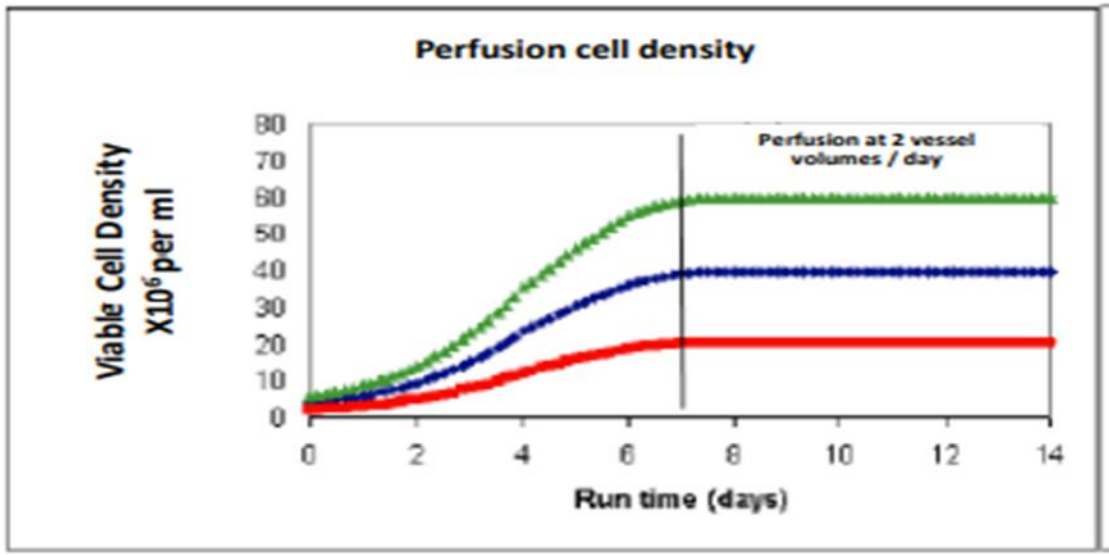


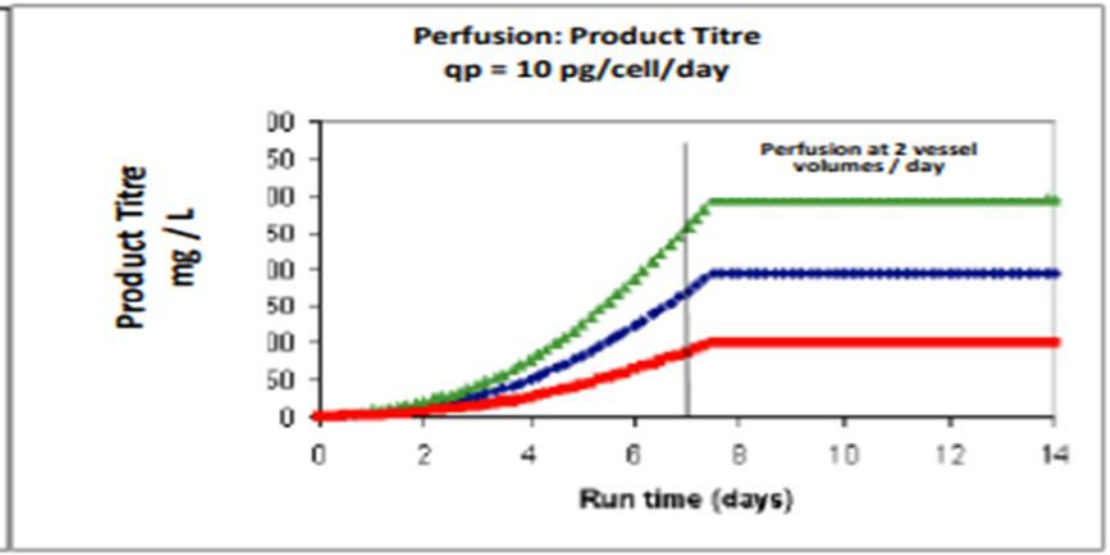
Peak day 8 cell density (million cells/ml)	volumetric productivity (mg/day/bioreactor liter) during a 14 day period	
	Fed-batch	Perfusion
20	81	200
10	41	100
2	8	20

~150% increase with perfusion

Productivity – Perfusion Model

A major advantage of perfusion is the ability to achieve increased peak cell densities. Densities as high as 100 x 10^6 cells/mL are possible



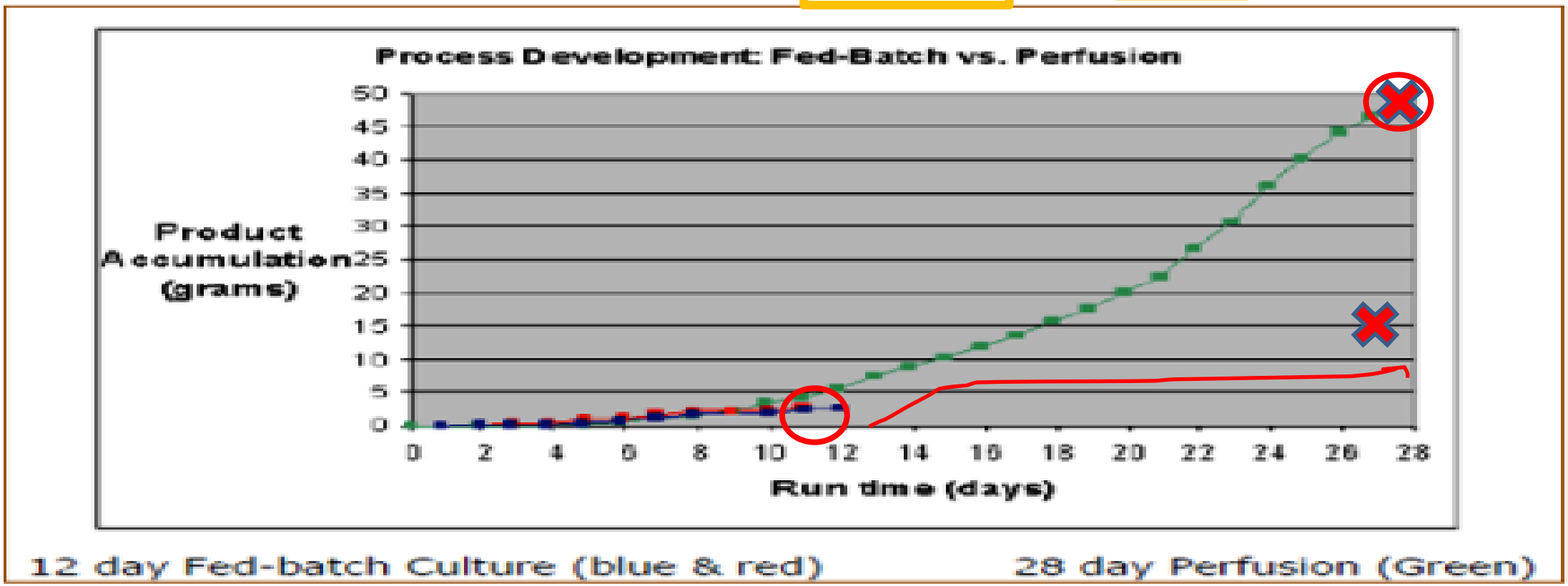


Steady state cell density (million cells/ml)	Steady state conditions			Product from a 14 day fed-batch 100L bioreactor* (grams)
	Product titer (mg/L)	Volumetric productivity at 2 VVD (mg/day/bioreactor liter)	Daily product accumulation from a 100L bioreactor (grams)	
20	100	200	20	114 (every 14 days)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

*peak density = 20 million cells/ml

Perfusion

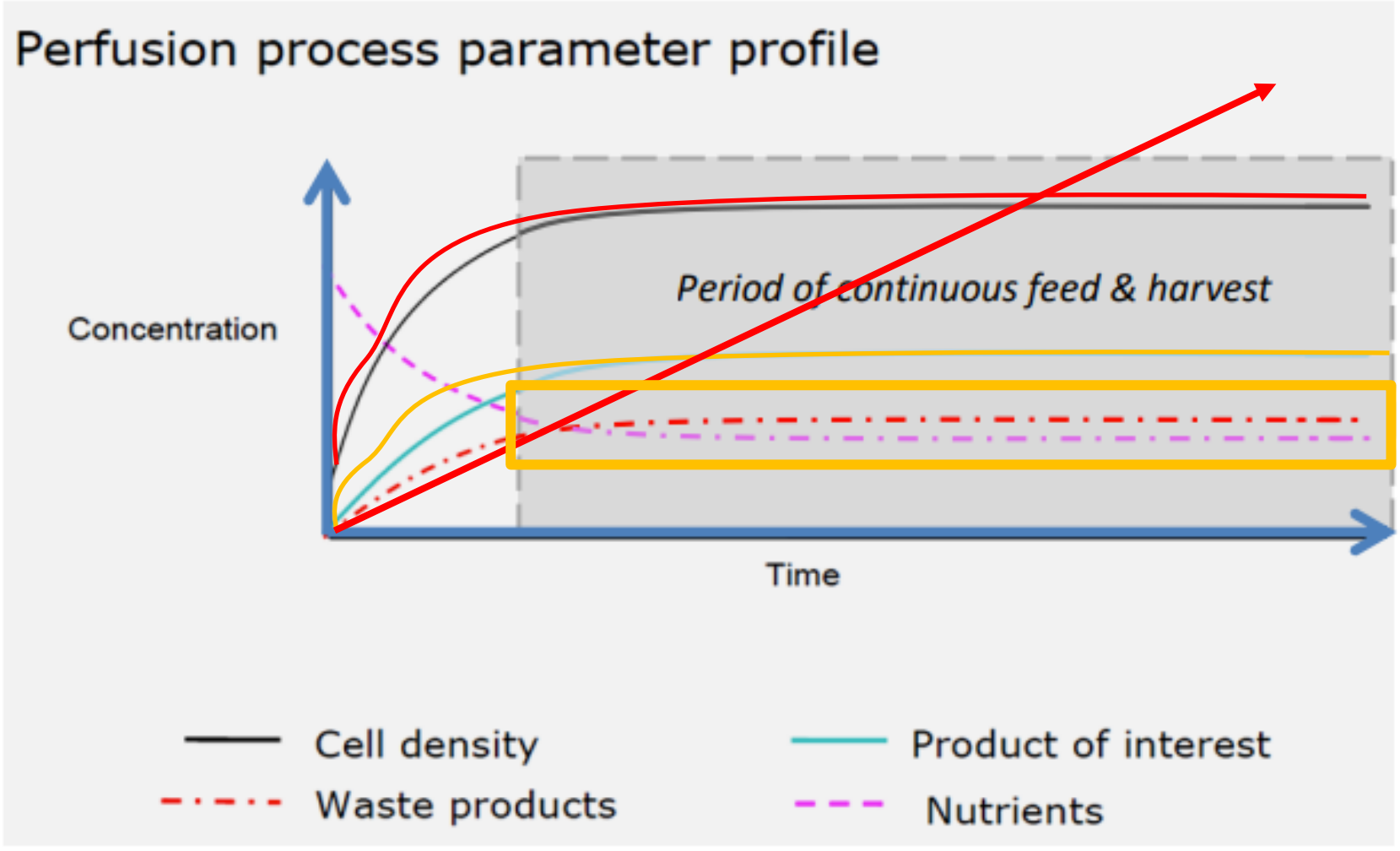
- **Fed batch culture**: circa 55 mg/L/day (12 days) with 2.8g total product
- **Perfusion culture**: circa 452 mg/L/day (28 days) with 50.0g total product



Perfusion Bioreactor Operation

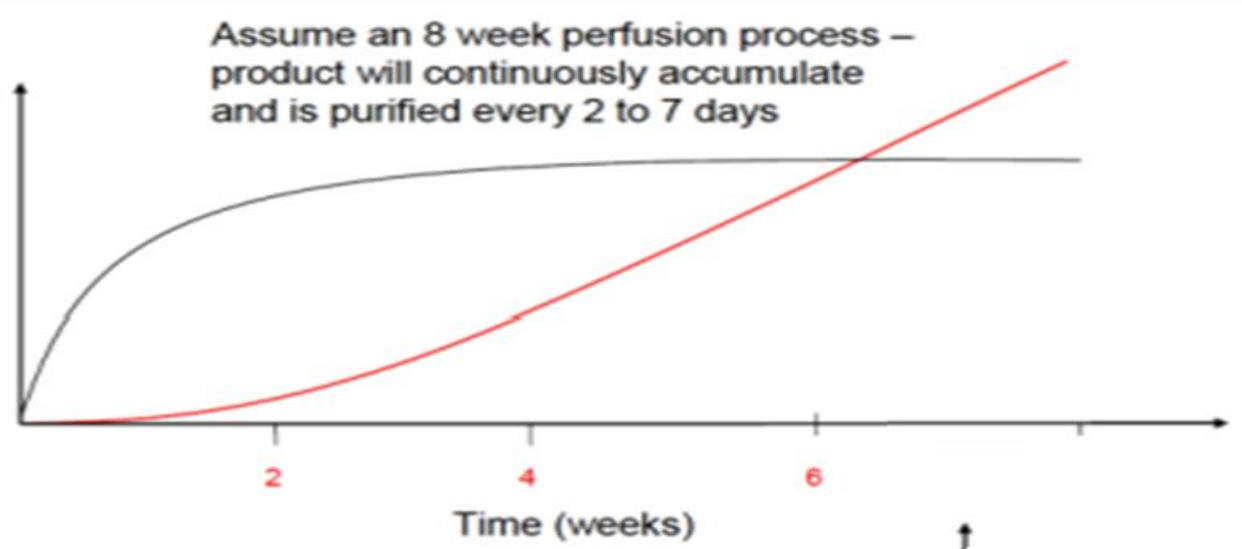
- The reactor is inoculated from a seed train as before
- During operation, fresh media (nutrient) is fed continuously into the bioreactor.
  - Batch delivery of media into the vessel is also possible linked to some predefined criteria (media pH, lactate levels, glucose levels etc.)
- Spent media (perfusate) containing product is harvested continuously via a cell retention device
  - Retains cells within the bioreactor to maintain a high cell density
  - The operating volume of the vessel is kept constant by feeding and harvesting at the same rate
- Duration of production run: typically 21 to 60+ days (some now running out to 120+ days)

Perfusion Bioreactor Operation Profile



From CMC Biologics online resource: Perfusion - Jeopardy or the Ultimate Advantage (2009) by Carstens, J.N., Clarke, H.R.G., and Jensen, J.P. http://www.cmcbio.com/Portals/0/CMC/docs/perfusion.pdf

Perfusion Bioreactor Operation Profile



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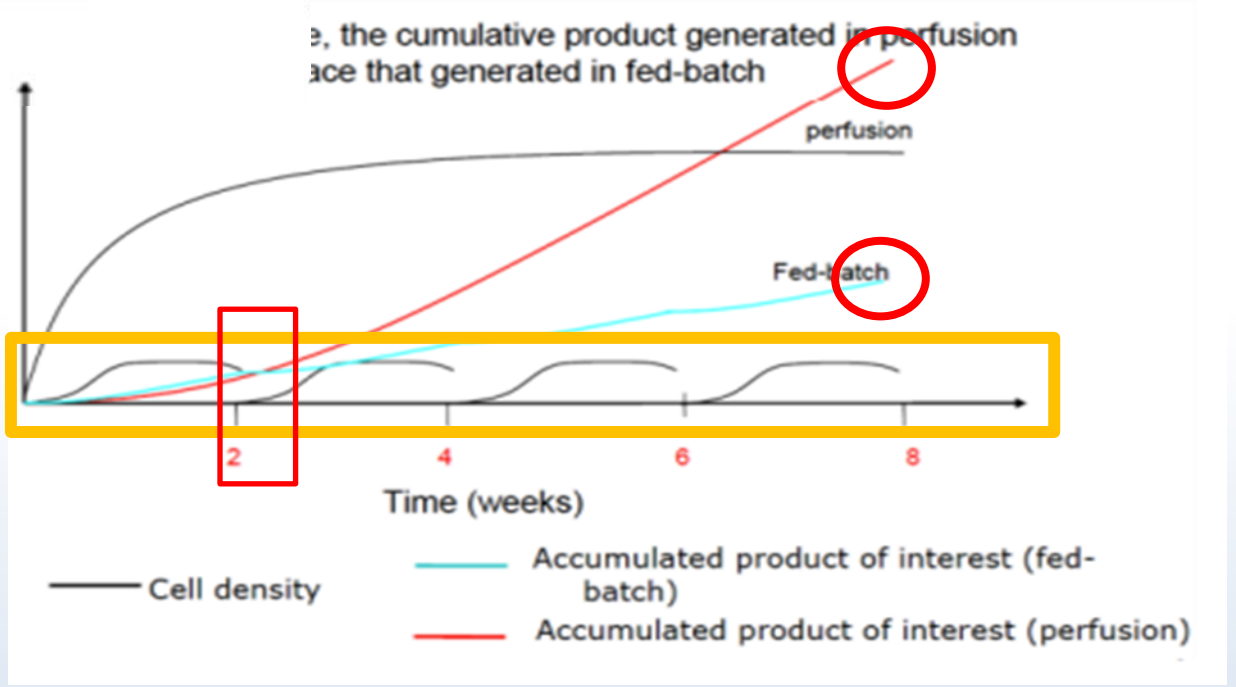
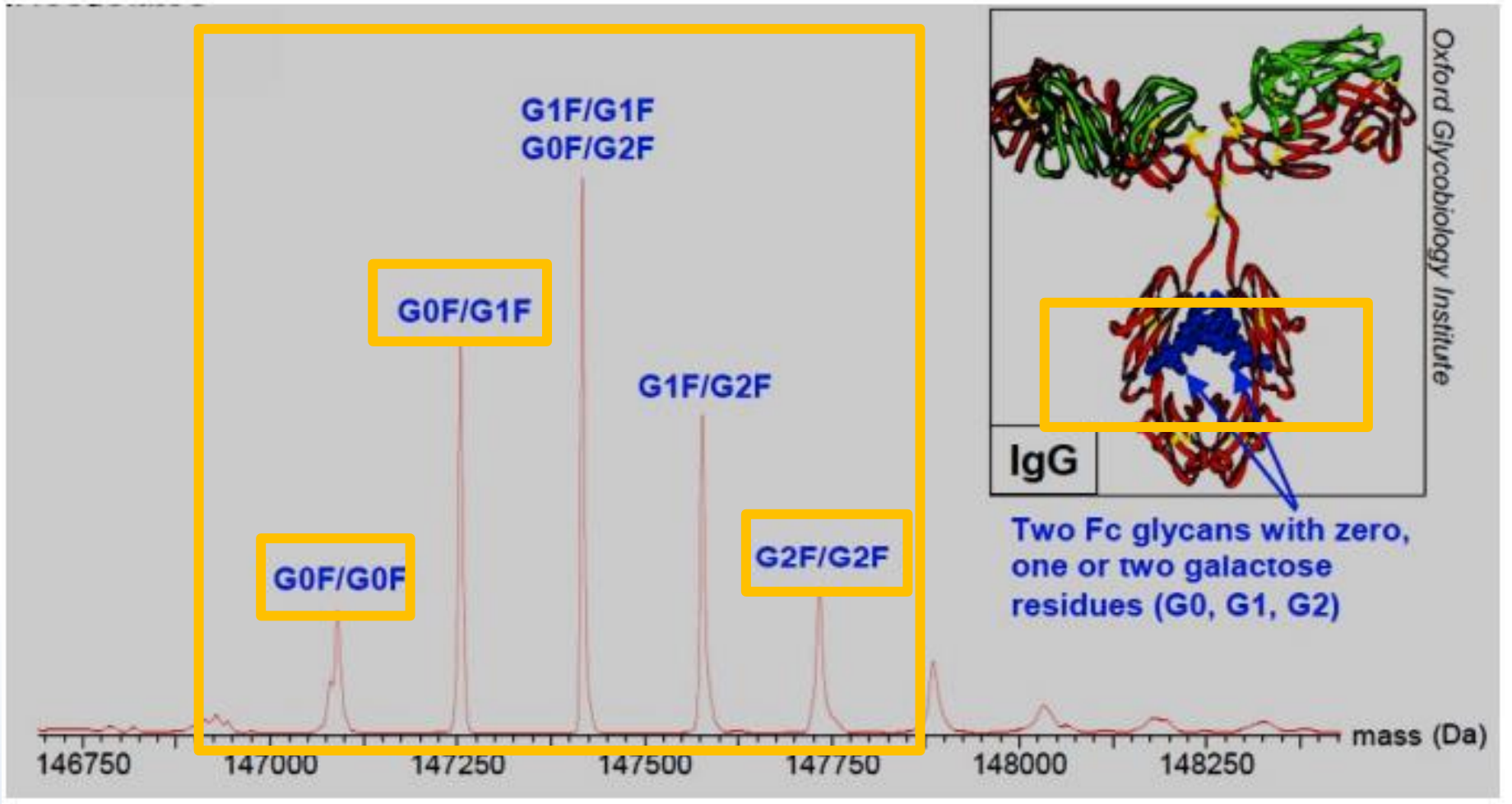


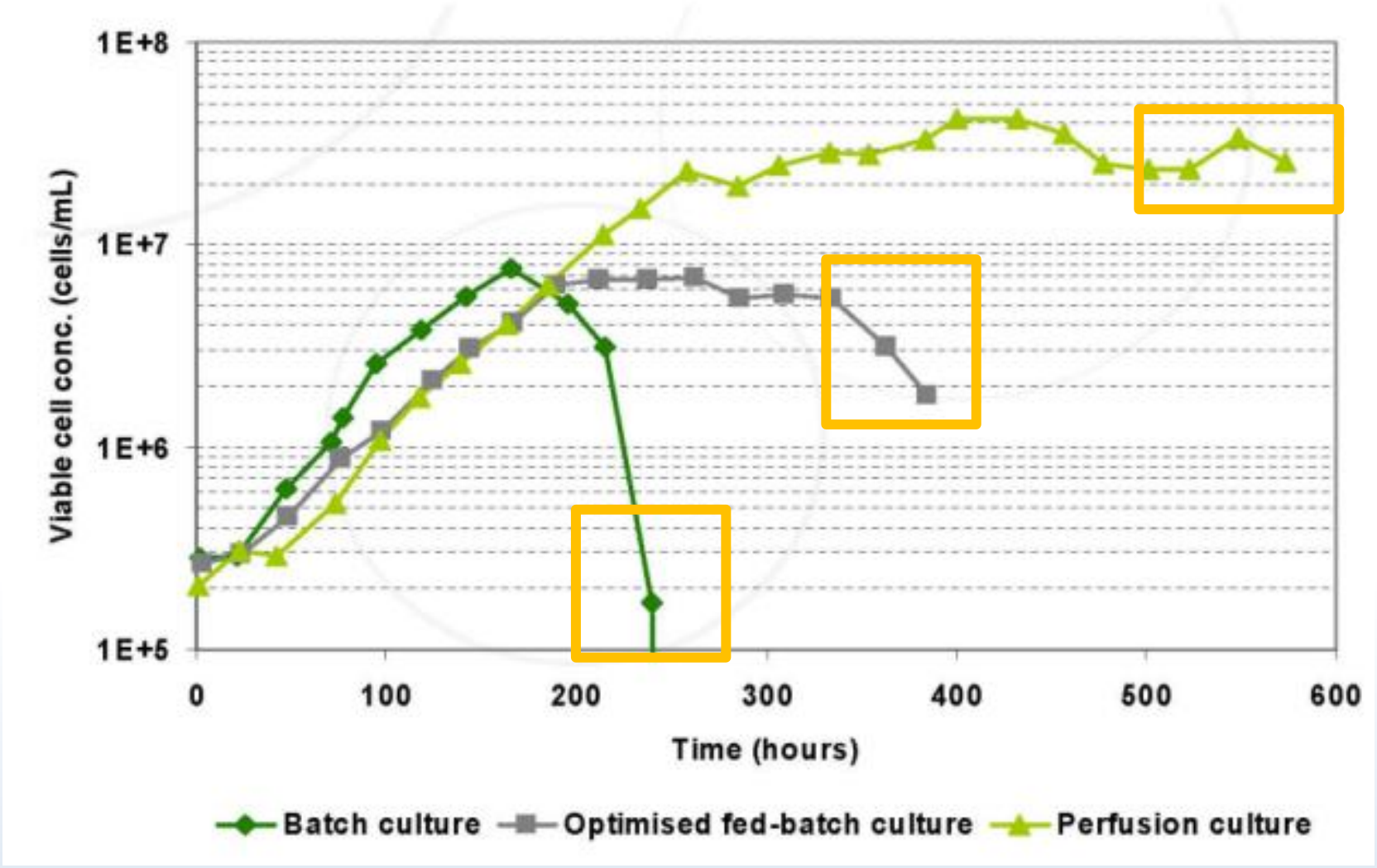
QA of Product

- Process validation must demonstrate the consistency of the entire process and monitor the product quality.
- Protein structural analysis:
  - SDS-PAGE and IEF
  - HPLC: SE, RP, IEX
  - Capillary electrophoresis
  - HPLC peptide mapping
  - Mass spectrometry – Maldi-TOF, LCMSMS: analysis of protein structure,, and other post-translational modifications
  - Glycan analysis
  - Circular dichroism
  - Functional assays – ELISA / Bioassay / BIAcore etc.

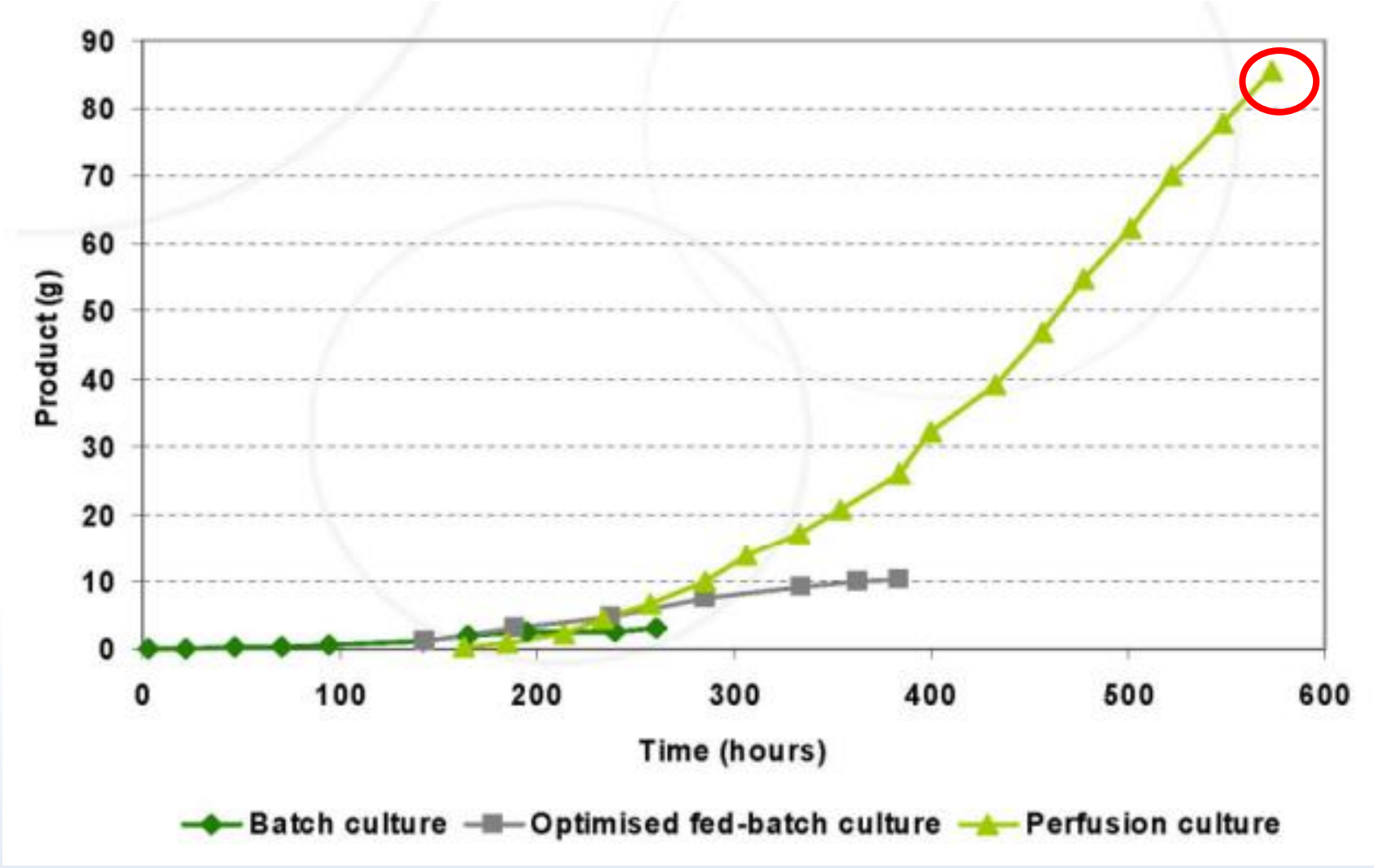
Quality Assurance of MAb Production



Typical Comparison of Cell Culture Performances



Typical Comparison of Cell Culture Performances Cont/d.

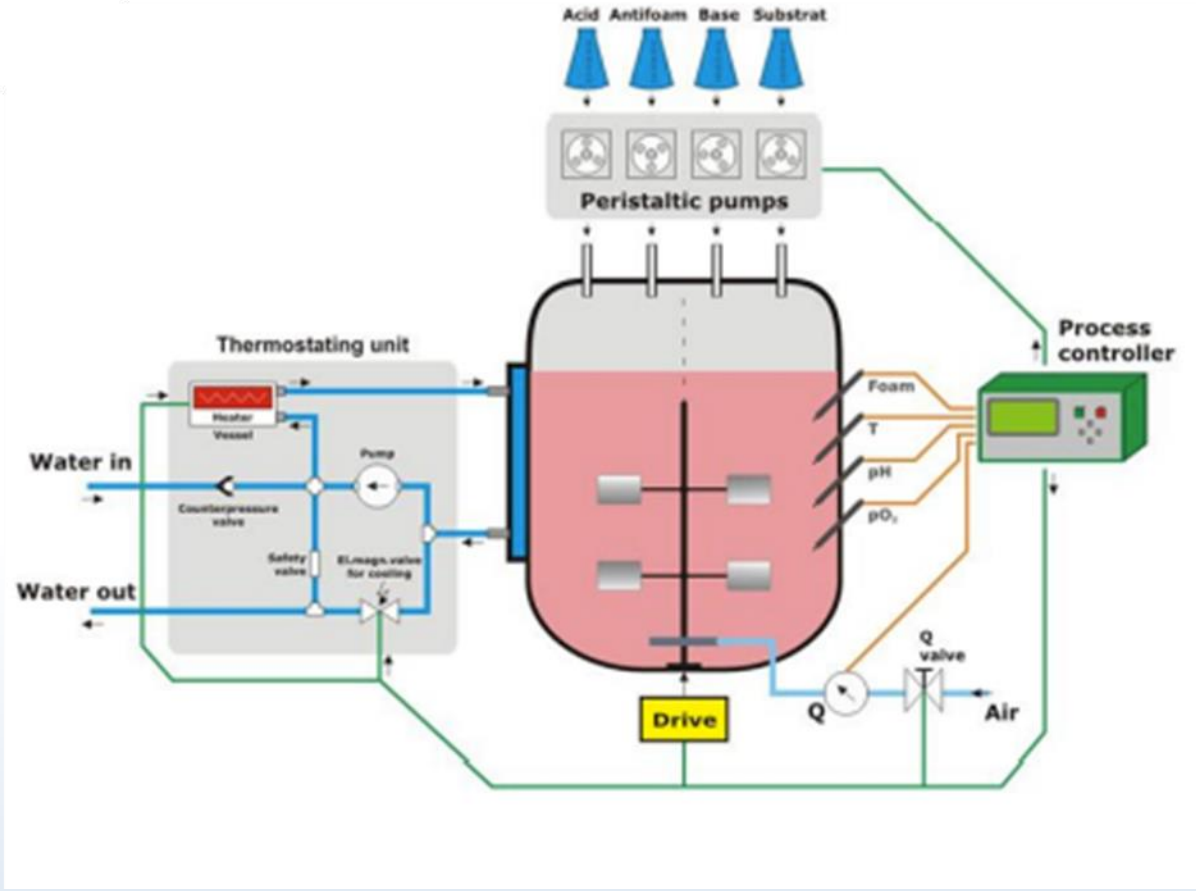


Comparison of batch, fed-batch and perfusion systems

	STBR (batch or fed batch	*Hollow fibre*	*Perfusion (spin-filter)*
Culture type	Suspension	Attached	Suspension
Cell conc.	Low/medium	**High**	Medium
Productivity	Low/medium	**High**	**High**
Specific cell productivity	Low/medium	Insufficient data	**High**
Scale-up	**Easy**	Very difficult	**Easy**
Operation	**Simple**	Complex	**Relatively simple**
Monitoring and control	**Direct and easy**	Indirect and complex	**Direct and easy**
Operating cost	**Low**	High	**Low**

Next.....

- Bioreactor controls – which parameters to control and how



Summary Points

- Bioreactors - provide controlled environments for the aseptic growth of cells and sterile product formation.
- Modes of operation include batch, fed-batch and perfusion systems with perfusion offering the best performance w.r.t. Cell density and product formation.
- Critical parameters to be controlled are temperature (36+0.5°C), pH (6.8 to 7.4), DO and cell number & viability.
- Use process control loops to monitor and control conditions in real time

Check out....

- Bioreactor Operational Excellence: Best Practices from Scale-up to Control By Brian J. Stamper and Cillian McCabe, BioProcess Research and Development, Eli Lilly and Company
- http://www.pharmamanufacturing.com/articles/2009/045.html?page=1

Questions?

Sample Questions

- Write a detailed note on perfusion operation of a bioreactor and the productivity it allows. What are the key advantages offered for the biopharma company?
- Compare and contrast batch and fed-batch bioreactor operations. Include comment on productivity, duration, feeding regimes, and cell densities.
- Fed-batch operation is still widely used in the biopharma industry. How does this work and what are the key control issues from an operational point of view?