Continuous BioManufacturing of Mabs by QbD

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Continuous Biomanufacturing of Therapeutic Mabs

Smaller equipment and facilities

- More flexible operation
- Reduced inventory
- Lower capital costs, less work-in-progress materials
- Smaller ecological footprint

Integrated processing with fewer steps.

- No manual handling, increased safety
- Shorter processing times
- Increased efficiency

On-line monitoring and control for increased product quality assurance in real-time (PAT)

- Amenable to Real Time Release Testing approaches
- Consistent quality

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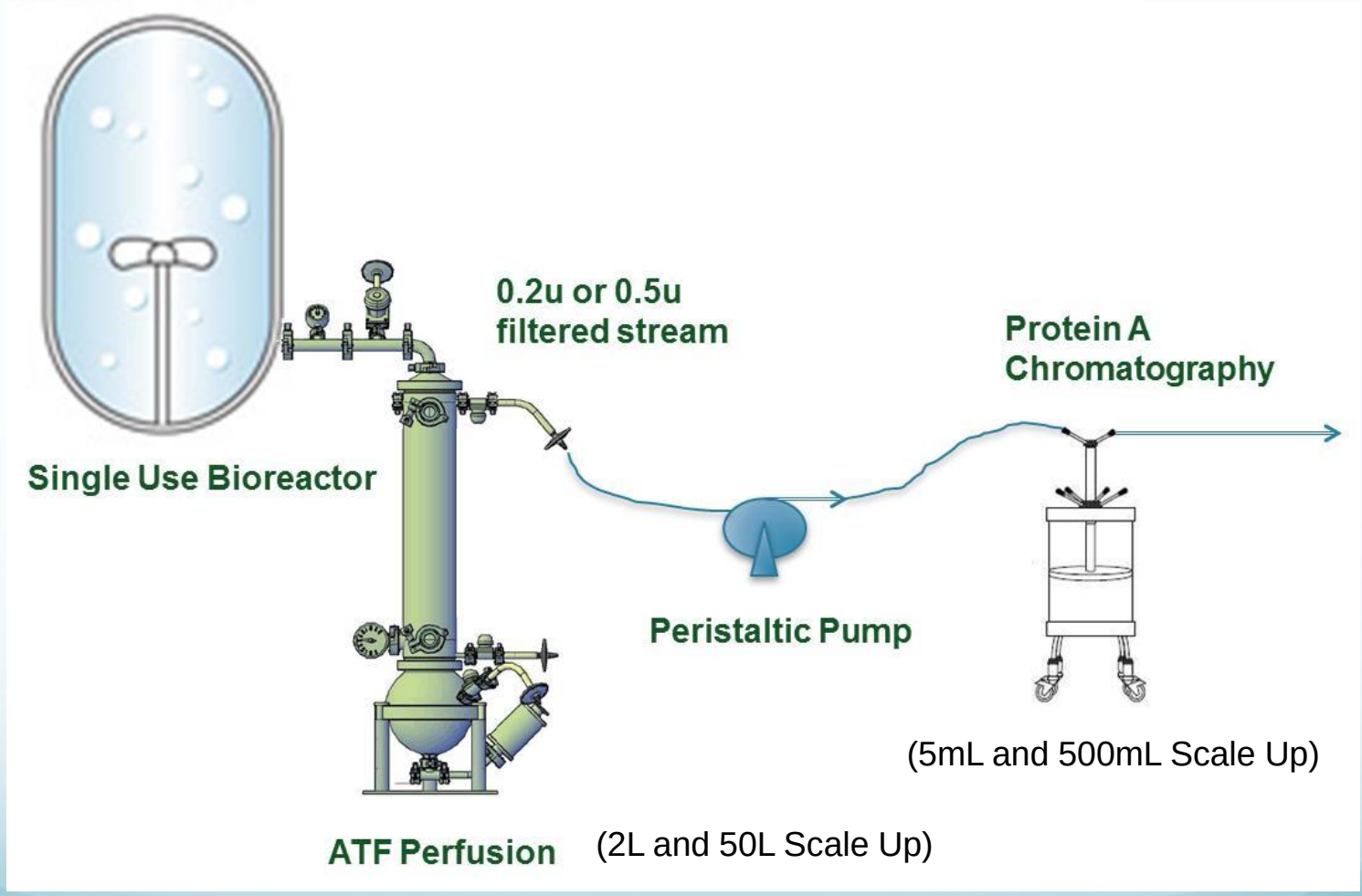
Continuous Manufacturing of Mabs Upstream and Protein A Downstream



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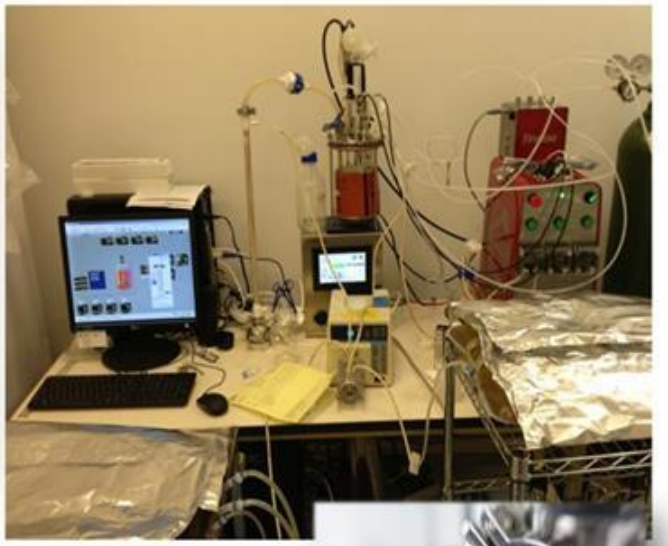
Our Continuous Biomanufacturing Pilot Plant



CQAs
(eg. Glycosylation, HCP, DNA, Aggregation)

- **Glycosylation (eg. Galactosylation, Fucosylation)**

IgG1	% HighMan	% G0	% G1	% G2	% Sialic acid	% Gal Alpha	% Core Fucose
A-Mab	7±5	71±10	19±10	3±10	1±5	0±5	91±20
B-Mab	1±5	78±10	17±10	4±10	2±5	0±5	94±20
R-Mab	2±5	46±10	40±10	12±10	3±5	0±5	95±20

CQA	Acceptable Range
HCP	0-100ng/mg
DNA	$<10^{-3}$ ng/dose
Aggregates	0-5%

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Risk Assessment Mitigation Matrix (RAMM) for selecting CPPs

Relative Importance of Output on CQA	9	1	1	9	3	3	3	3	3	3			
Process Parameters	pH	CO2	VCD	Viability	Bioburden	Endotoxin	Titer	Mycoplasm	In Vitro viral	MMV PCR	Tot	Impacts	Proc
Perfusion Rate	1	1	9	9	1	1	1	1	1	1	127	Quality & Growth	WC-CPP
Sparge Oxygen Flowrate	1	9	1	1	1	1	1	1	1	1	55		
Agitation 110-125 RPM	3	9	3	3	1	1	3	1	1	1	99	Gas Evolution Rate	WC-CPP
pH	0	3	3	3	1	1	3	1	1	1	138	Quality & Growth	CPP
DO (>40%)	1	1	3	3	1	1	3	1	1	1	73	Titer	
Seed Density (0.5 x 10E6)	1	1	3	9	1	1	3	1	1	1	127	Cell Growth	WC-CPP
N-1 Inoculum Volume	1	1	1	1	1	1	1	1	1	1	47		
Temperature	1	1	3	9	1	1	9	1	1	1	163	Quality &Growth	CPP
Duration of Perfusion	1	1	1	3	1	1	1	1	1	1	83		
Glucose Control	1	1	9	9	1	1	9	1	1	1	151	Quality & Growth	CPP
Lactate Control	1	1	9	9	1	1	9	1	1	1	151	Quality & Growth	CPP
Anti-Foam Control	3	1	1	1	1	1	1	1	1	1	65		
Protein Load Protein A	1	1	1	1	3	3	9	3	1	1	161	Quality & Titer	CPP
Elution pH Protein A	1	1	1	1	3	3	9	3	1	1	161	Quality & Titer	CPP
Totals	17	32	48	62	18	18	62	18	14	14			

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Design of Experiments (DoE) (Taguchi L9)

Settings	Temperature (°C)	pH	Glucose
1	36.5	7.2	0
2	36.5	7	1
3	36.5	6.8	3
4	36	7.2	1
5	36	7	3
6	36	6.8	0
7	35.5	7.2	3
8	35.5	7	0
9	35.5	6.8	1

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Continuous Culture Overview

- It can produce high titers and good quality product
- Equipment is utilized more efficiently and the equipment footprint is much smaller
- The Continous System pushes productivity to the next level, ~10x higher cell densities

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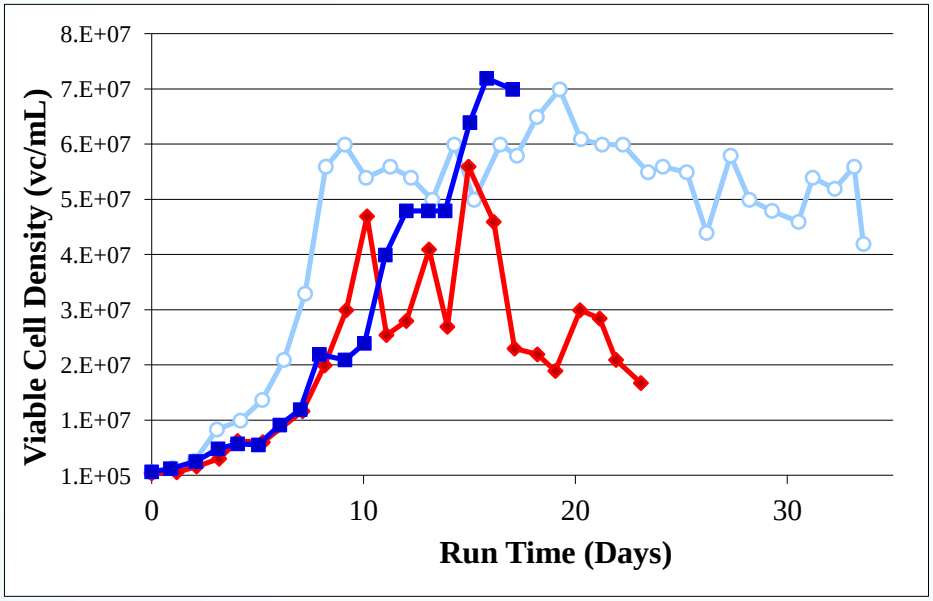
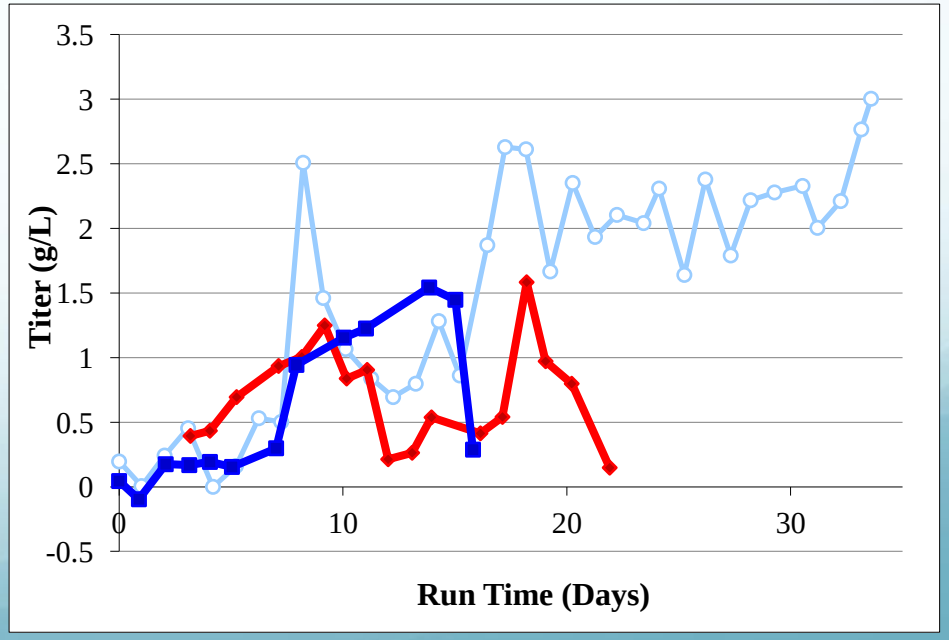
Upstream Continuous Cell Culture



Key

- Run 1
- Run 2
- Run 3



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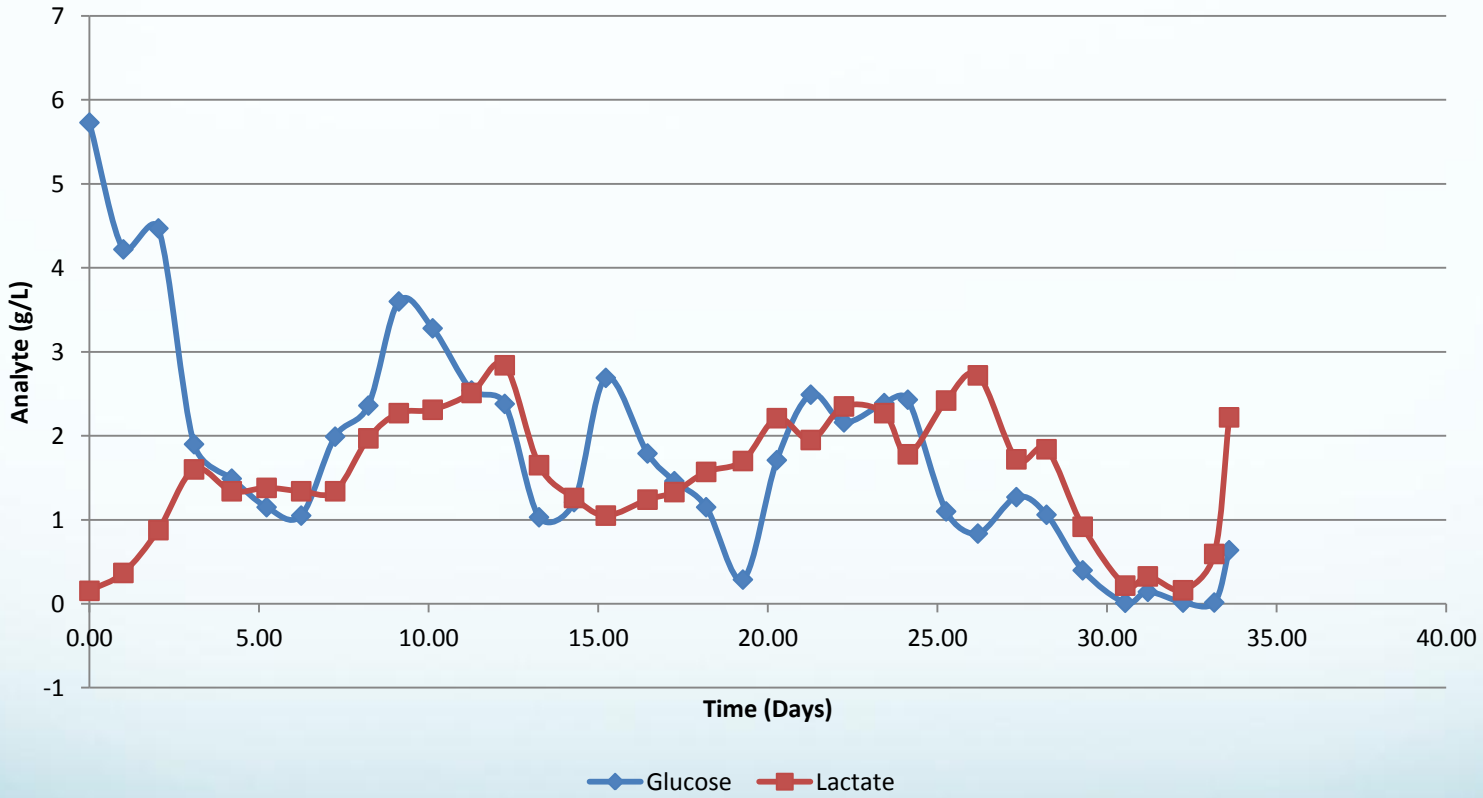
Glucose and Lactate Control



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Design Space (DS) based on Glycosylation CQA

IgG1	% HighMan	% G0	% G1	% G2	% Sialic acid	% Gal Alpha	% Core Fucose
A-Mab (Test Article)	0	54	39	7	2	0	97
A-Mab (Reference)	7±5	71±10	19±10	3±10	1±5	0±5	91±20
B-Mab (Test Article)	0	85	15	1	0	0	96
B-Mab (Reference)	1±5	78±10	17±10	4±10	2±5	0±5	94±20

Downstream Continuous Purification

- Synchronize the protein load (g/mL of resin) with the upstream rate of perfusion
- Replace interim holding tanks with peristaltic pumps to perform continuous downstream purification
- Reduce the amount of Protein A resin by ~ 20-fold

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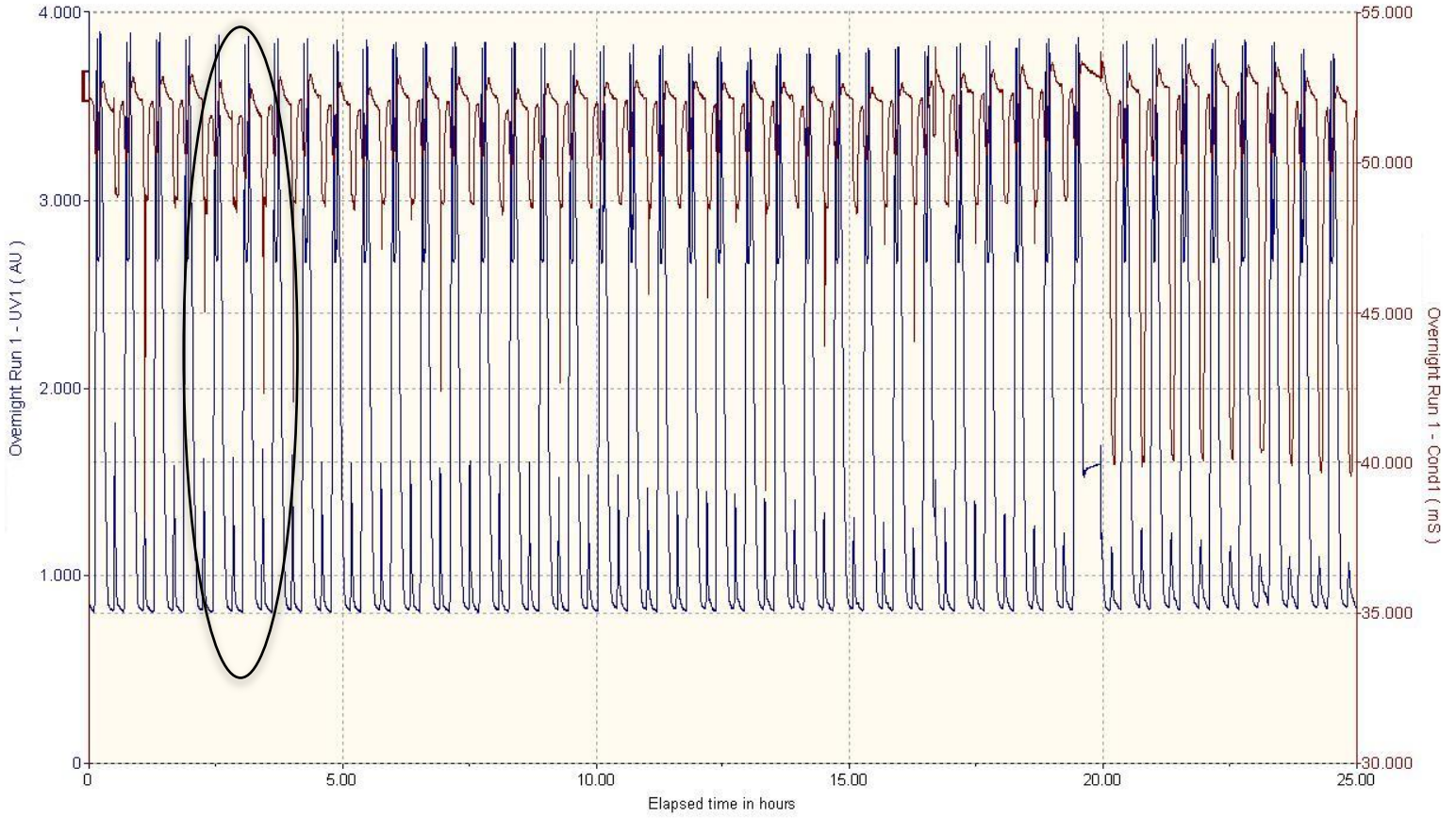
Continuous Purification of A-Mab



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Antibody production

Perfusion	Per Day *	60-Day Run	6 Runs/Yr
50L	25g	1.5Kg	9Kg
1000L	500g	30Kg	180Kg

Fed-Batch	Per Run *	20 Runs/Yr
50L	25g	0.5Kg
1000L	500g	10Kg

* 50% Purfication Yield

18X More Product in the same time

Assumptions

Parameter	FB	CP
Production Bioreactor	10,000L	1,000L
Product Titer	2 g/L	2 g/L
Production Phase	10 days	10 days (1VVD)
Media Consumption	**10,000L**	**10,000L**
Protein A Resin **($11,400/L)**	**40L ($456,000)**	**2L ($22,400)**

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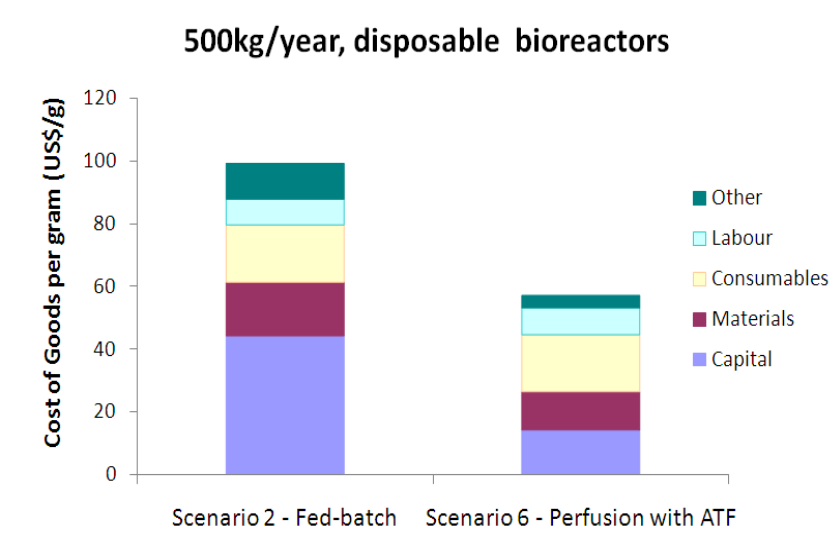
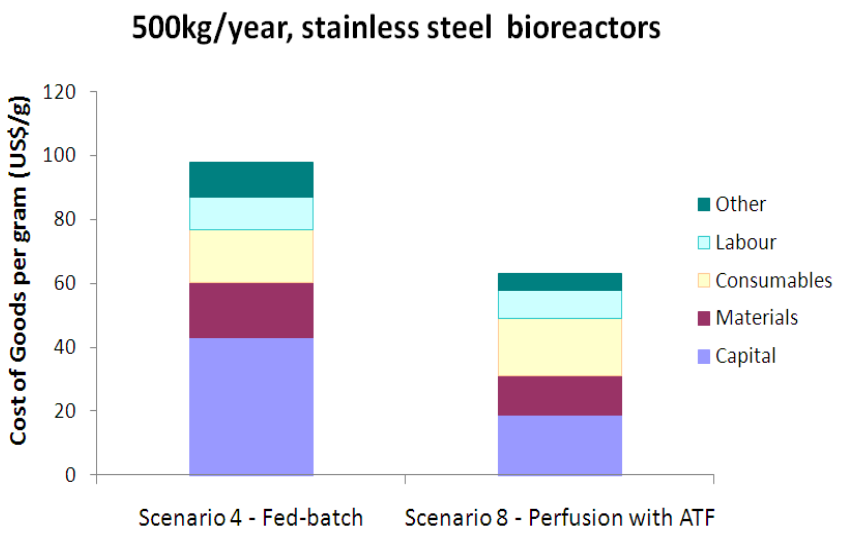
Results at 500Kg / year

- A comparison of fed-batch to perfusion at “large” scale:
  - Perfusion utilizes a smaller footprint and
  - Lower capital investment costs
  - And has lower operating costs
- The advantage of disposable bioreactors reduces with increasing bioreactor size, as required for large scale fed-batch operation for example





Capital (US$)	106.7M	33.1M
Floor Area (m^2)	4,012	2,447

Capital (US$)	103.9M	44.1M
Floor Area (m^2)	5,096	3,152

KEY POINTS

- Making therapeutic antibodies can benefit from new advances in continuous biomanufacturing such as Continuous Perfusion (CP) and Continuous Chromatography (CC).

- The Capex for a new facility is significantly lower than conventional batch facilities