12 October 2009 BPI Meeting, Raleigh, NC

Economic Models Guiding Expression System Choices in Early Phase Clinical Development

PROMEDIOR

therapeutics to treat fibrosis

Promedior Overview

- Startup biopharmaceutical company developing therapeutics to treat fibrosis
- Located in Malvern, PA with 12 employees
- Research dedicated to understanding the biology of fibrosis
- Development currently focused on first drug candidate, PRM-151
- Manufacturing and testing conducted externally by contract organizations
- Phase I clinical safety trial initiated in July, 2009
- First Phase II clinical study scheduled for 2010

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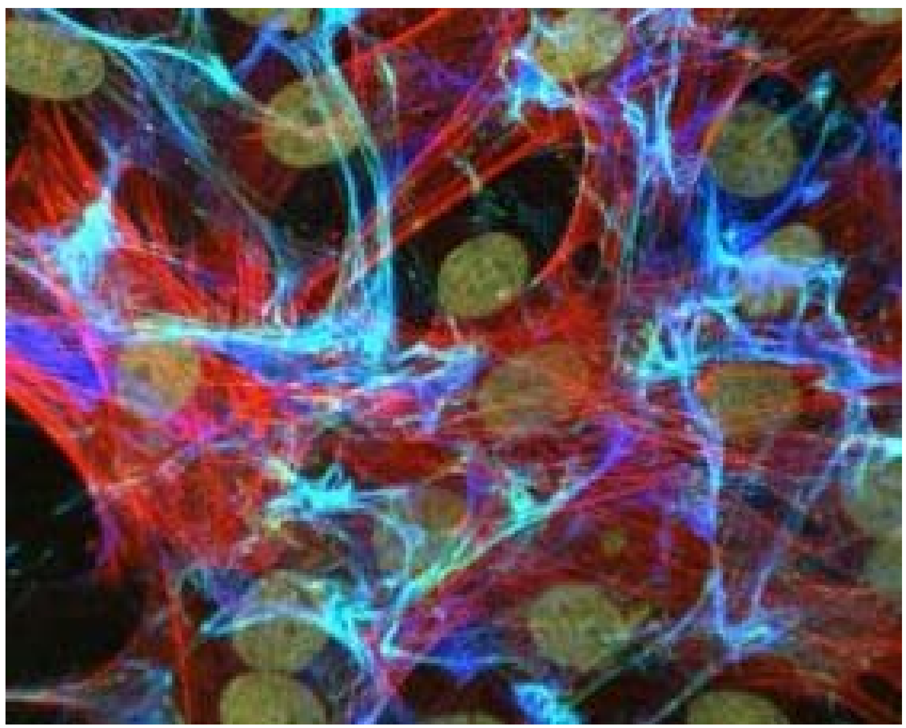
Fibrosis Overview

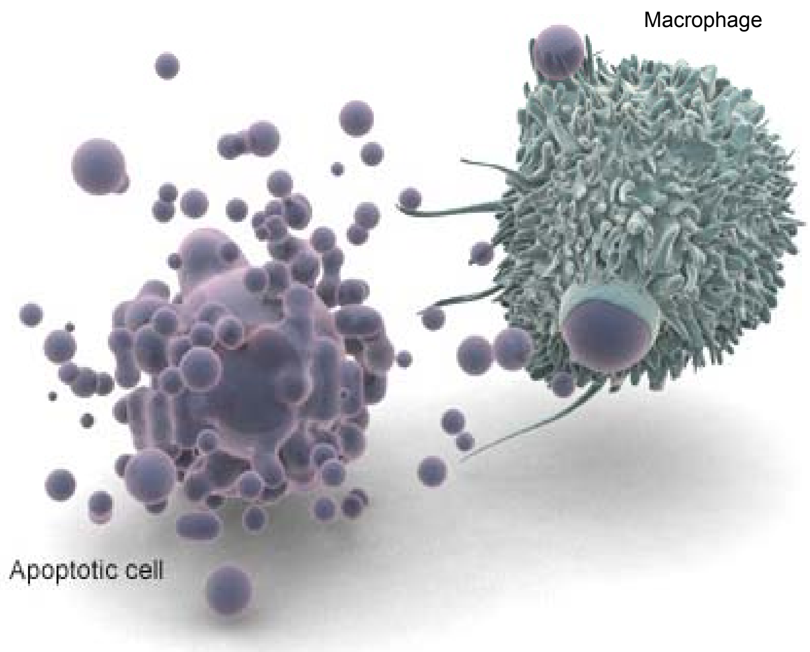
- Fibrosis is the pathologic accumulation of extracellular matrix proteins in tissue
  - Resulting from abnormal stimulation of two specific cell populations - monocytes and myofibroblasts.
- Fibrosis is a major cause of significant morbidity and mortality in many disease indications, with limited treatment options.



Collagen I
Myofibroblast

PRM-151: Recombinant Human Serum Amyloid P

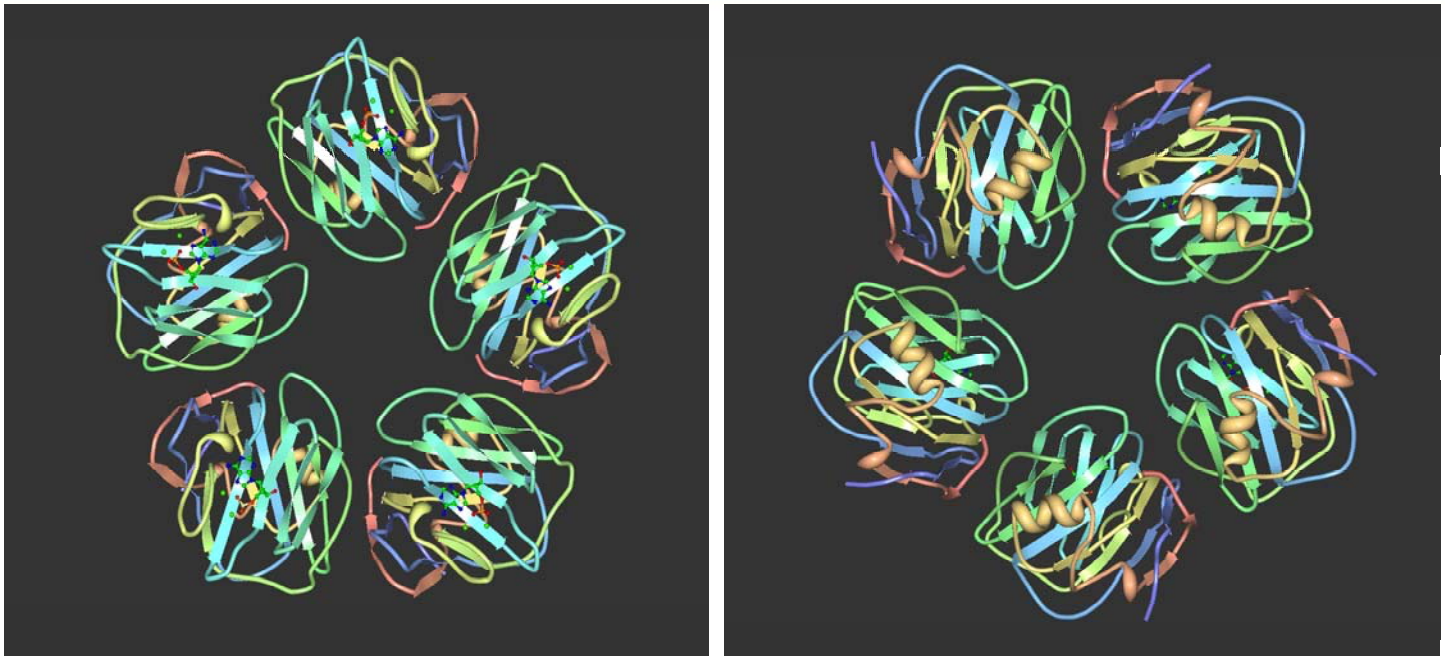
- Member of the pentraxin family of proteins (CRP, PTX3)
- Highly conserved sequence and function across species
- Circulates at a constant 30 μg/mL in plasma
- Soluble pattern recognition receptor; signals through FcγR
- Potent modulator of monocyte differentiation *in vitro and in vivo*
- Broad anti-fibrotic activity across tissues, models and species



U.S. National Library of Medicine

PRM-151: Biophysical Overview

Calcium-binding face



FcγR binding face

(PDB file 1LGN; Hohenester, E., et al, 1997, JMB 269(4):570-8)

- Non-covalent pentamer; 2 salt bridges/interface + VDW interactions
- 204 aa /protamer – 125 kDa pentamer
- 1 intramolecular disulfide bond
- 1 N-linked biantennary glycosylation site/protomer, up to 10 SA/pentamer
- 2 Calcium binding sites/protomer = 10 Ca^{+2} sites/pentamer on one face

PRM-151: Project Background

- Began with no expression system, analytics, or process
  - Literature reported expression in *P. Pastoris, E. coli*
  - Literature also reported purification from human plasma
- Strategy was to pursue 3 tracks in parallel
  - Microbial expression in E. coli: IB refold & periplasmic
  - Mammalian cell line development, secreted
  - Source from human plasma, or plasma fraction
- Initial progress
  - Quick attempts at refolding inclusion bodies unsuccessful
  - Little to no soluble expression in cytosolic or periplasmic fractions
  - Several technologies for mammalian expression unsuccessful
  - Difficulty sourcing plasma fractions to develop recovery from human plasma

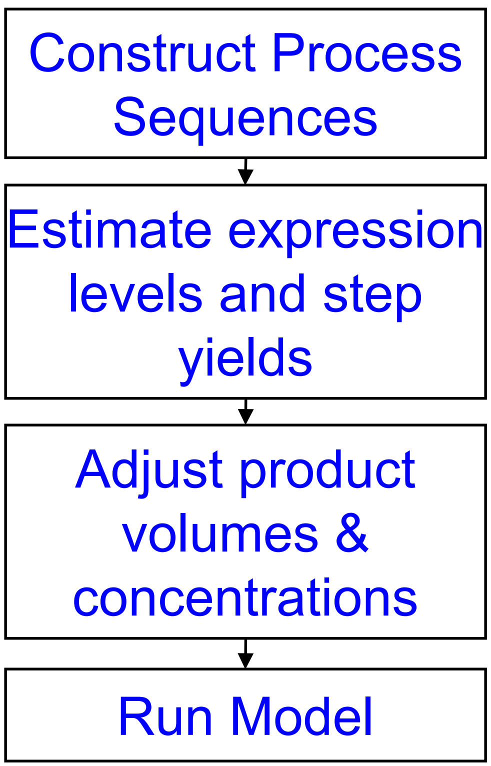
PRM-151: Early Development Cost Modeling

What is the value of cost modeling early in development?

- Generates relative cost scenarios quickly and easily
- Provides useful comparisons of process alternatives
- Identifies process design/performance issues
- Frames process development decisions, highlights future impact

Biopharm Services BioSolve Cost Model

- Using BioPharm Services configurable BioSolve cost model v1.1.1
  All cost analysis calculations and plots are © Biopharm Services
- Screen potential process alternatives
  BPS scenario and sensitivity analyses facilitate process comparisons



- Use typical process sequences and parameters
- Use early process data when available
- Adjust process model to create plausible base case

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Constructing Process Models & Analyses

- Four Process Models
  - CHO Secreted
  - Microbial Inclusion Bodies: Refold by dilution
    - Detergent solubilization/Tris Buffer Dilution
    - GdHCl solubilization/Arginine Buffer Dilution
  - Microbial Periplasmic Secretion
  - Natural Source Human Plasma

Process Model Base Case
- Process Yield
- Production Schedule
- Batch size/throughput

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COGs Analysis
- $/gram
- Cost Breakdown

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Scenarios
- 200 L vs 10,000 L
- ± Disposables

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Sensitivities
- Bioreactor Vol
- Product Titer
- # Bioreactors
- % Capacity Utilization

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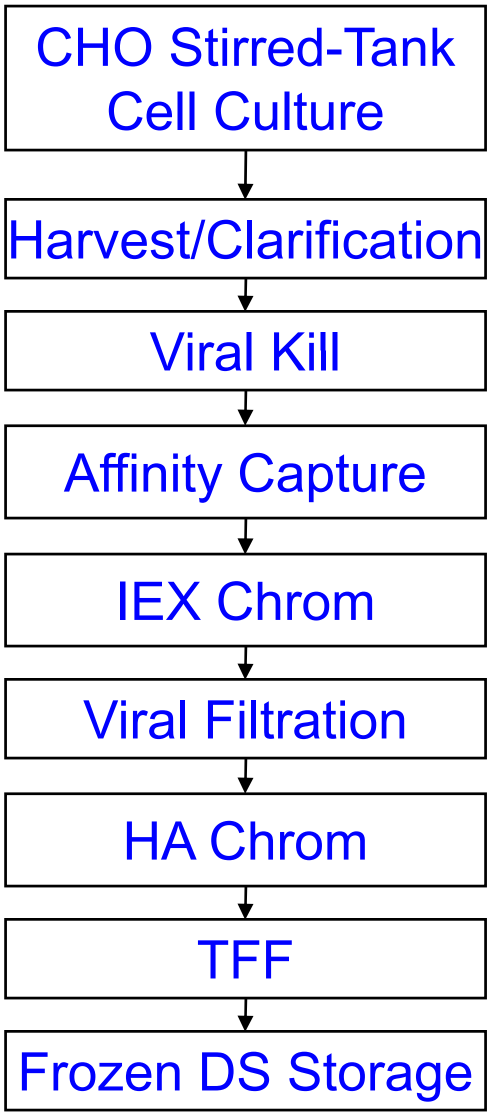
Process Comparisons

CHO Process

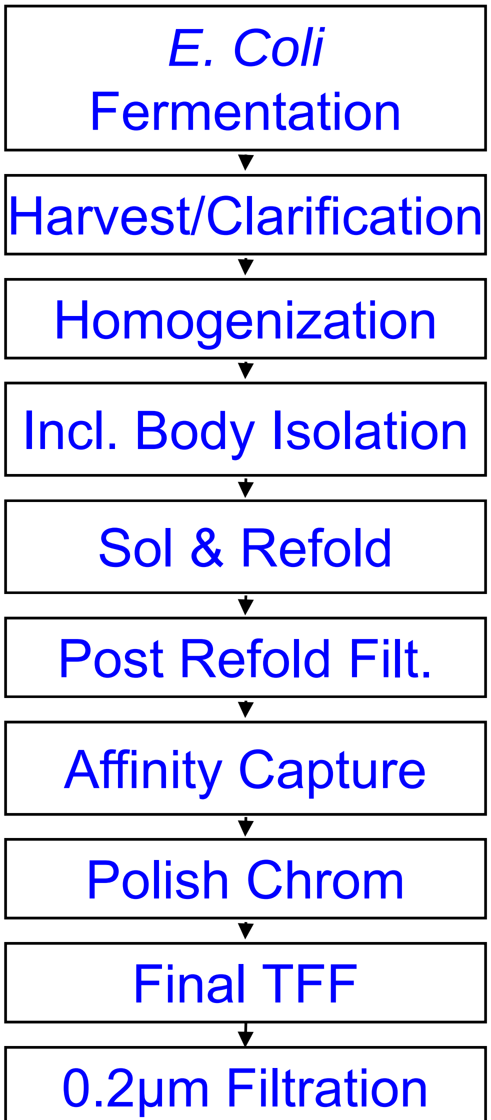


- A version of this process produced tox and clinical material at 200 L scale
- Product titer at harvest = 0.9 g/L
- Overall process yield = 32%
- 3 column steps needed for purification of desired glycoforms

	Process Sequence	Conc (g/L)	Vol (L)	Mass (g)	Yield (%)
1	N-2 Batch Seed Culture	0.0	5	0	100%
2	N-1 Fed-Batch Seed Culture	0.0	31	0	100%
3	Fed-Batch Production Culture	0.9	200	172	100%
4	PRM-151 Harvest/Clarification	0.9	187	163	95%
5	PRM-151 Virus Inactivation	0.8	206	155	95%
6	PRM-151 Affinity Capture	2.3	62	140	90%
7	PRM-151 IEX Chrom	9.3	10	89	64%
8	PRM-151 Viral Filtration	5.4	16	87	97%
9	Polishing Chrom	1.7	33	56	65%
10	30kDa TFF	20.8	3	56	99%
11	PRM-151 0.2µm Filtration	20.6	3	55	99%

Process Yield = 32%

Microbial Refold Process

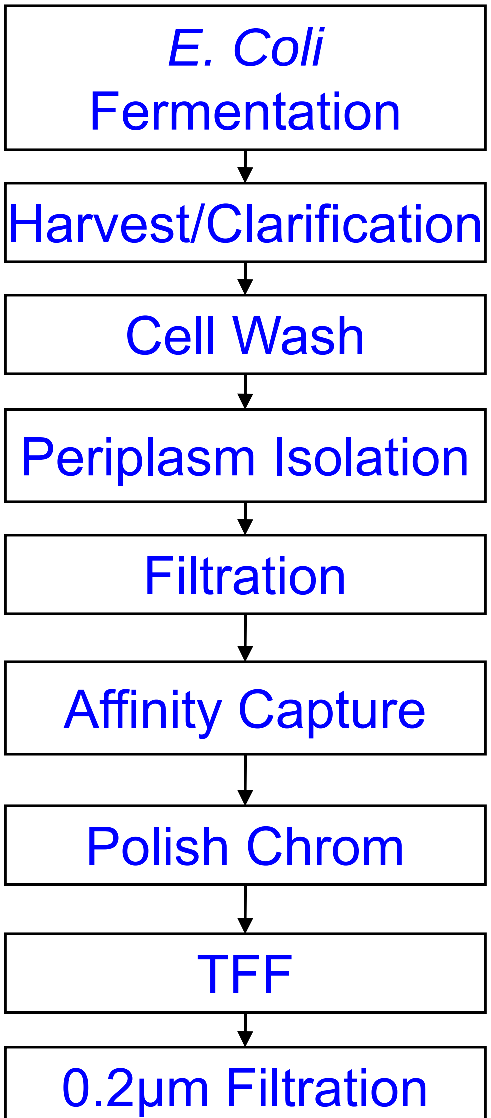


- Robust inclusion body expression
  - 2-8 g/L in 5 L fermentors
- Very insoluble IB's
  - 6M GdHCl or detergent needed to solubilize
- Assume refold by 10x dilution (crux of process)
- Assume only two chrom steps needed
- Assume 5 g/L base case with 12% process yield

	Process Sequence	Conc (g/L)	Vol (L)	Mass (g)	Yield (%)
1	rhSAP Seed Fermentor	0.000	20	0	100%
2	rhSAP Production Fermentor	5.0	200	1,000	100%
3	rhSAP Cell Harvest	22.5	40	900	90%
4	rhSAP Homogenisation	18.4	44	810	90%
5	rhSAP IB Isolation	33.1	22	729	90%
6	rhSAP IB wash	27.1	24	656	90%
7	rhSAP IB Sol & Refold	0.3	484	164	**25%**
8	rhSAP Post refold Filtration	0.3	484	156	95%
9	rhSAP Affinity Capture	3.7	38	140	90%
10	rhSAP AEX Chrom	8.9	14	126	90%
11	rhSAP TFF	20.6	6	124	98%
12	rhSAP 0.2m Filtration	20.2	6	121	98%

Process Yield = 12%

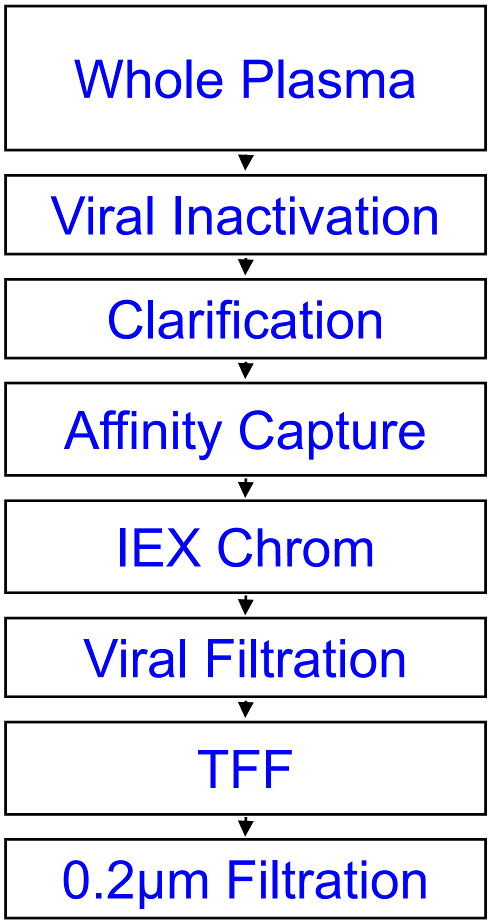
Microbial Periplasmic Secretion Process



- Assume expression as soluble pentamer
  - 0.25 g/L titer base case
  - 40% process yield
- Assume periplasmic isolation is scalable
- Assume only two chrom steps needed
  - No glycoform purification required

	Process Sequence	Conc. (g/L)	Vol (L)	Mass (g)	Yield (%)
1	rhSAP Seed Fermentor	0.0	20	0	0%
2	rhSAP Production Fermentor	0.25	200	50	0%
3	rhSAP Cell Harvest	1.1	40	45	90%
4	rhSAP Cell wash	0.9	44	41	90%
5	rhSAP Periplasmic Isolation	0.1	220	30	75%
6	rhSAP Depth Filtration	0.1	220	26	85%
7	rhSAP Affinity Capture	1.3	18	23	90%
8	rhSAP AEX Chrom	6.8	3	21	90%
9	rhSAP TFF	20.6	1	20	98%
10	rhSAP 0.2m Filtration	20.2	1	20	98%
				Process Yield =	40%

Human Plasma Process



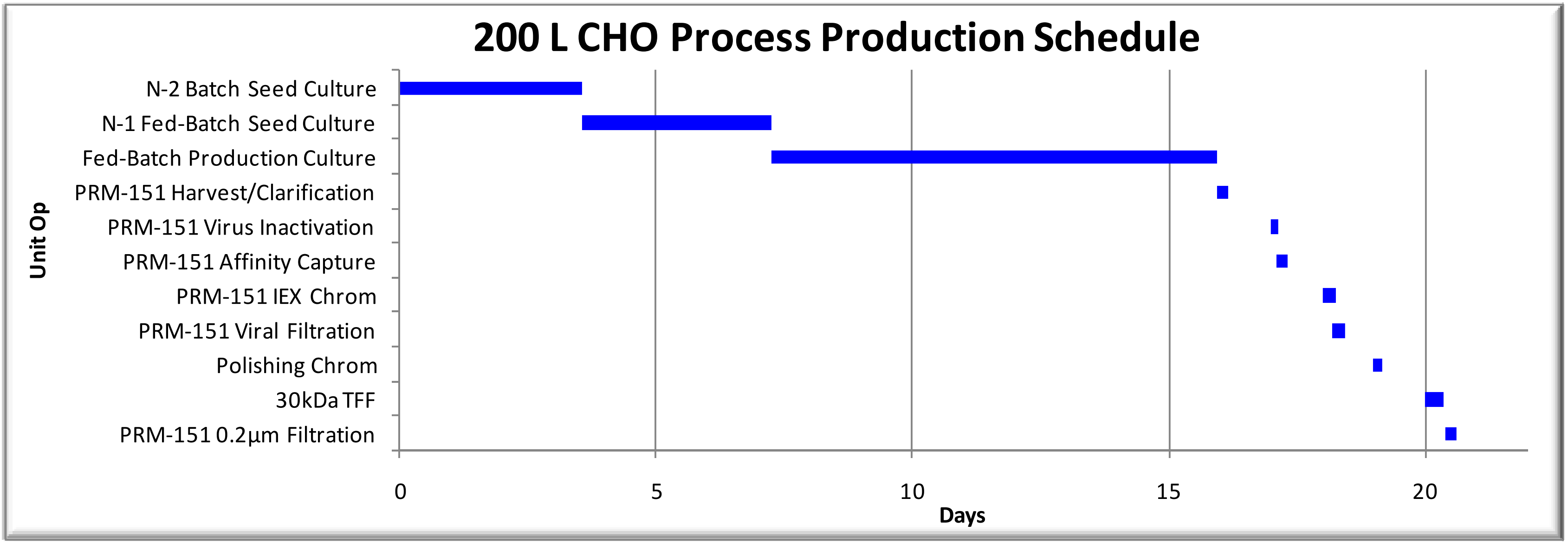
- Biggest issue is securing source of natural feedstock
  - whole plasma typically contains 0.03 g/L
  - plasma fraction (Cohn) – extraction at <0.03 g/L
- Assume whole plasma cost is ≥ $500/L
- 2 chrom steps
- 2 viral clearance steps
- Modeled high process yield = 72%

	Process Sequence	Conc (g/L)	Vol (L)	Mass (g)	Yield (%)
1	PRM-151 Virus Inactivation	0.027	220	5.9	98%
2	hSAP Affinity Capture	0.3	18	5	90%
3	hSAP AEX Chrom	1.5	3	5	90%
4	hSAP Viral Filtration	1.5	3	5	98%
5	hSAP UF/DF	20.9	0.2	4	95%
6	hSAP 0.2µm Filtration	20.5	0.2	4	98%

Process Yield = 72%

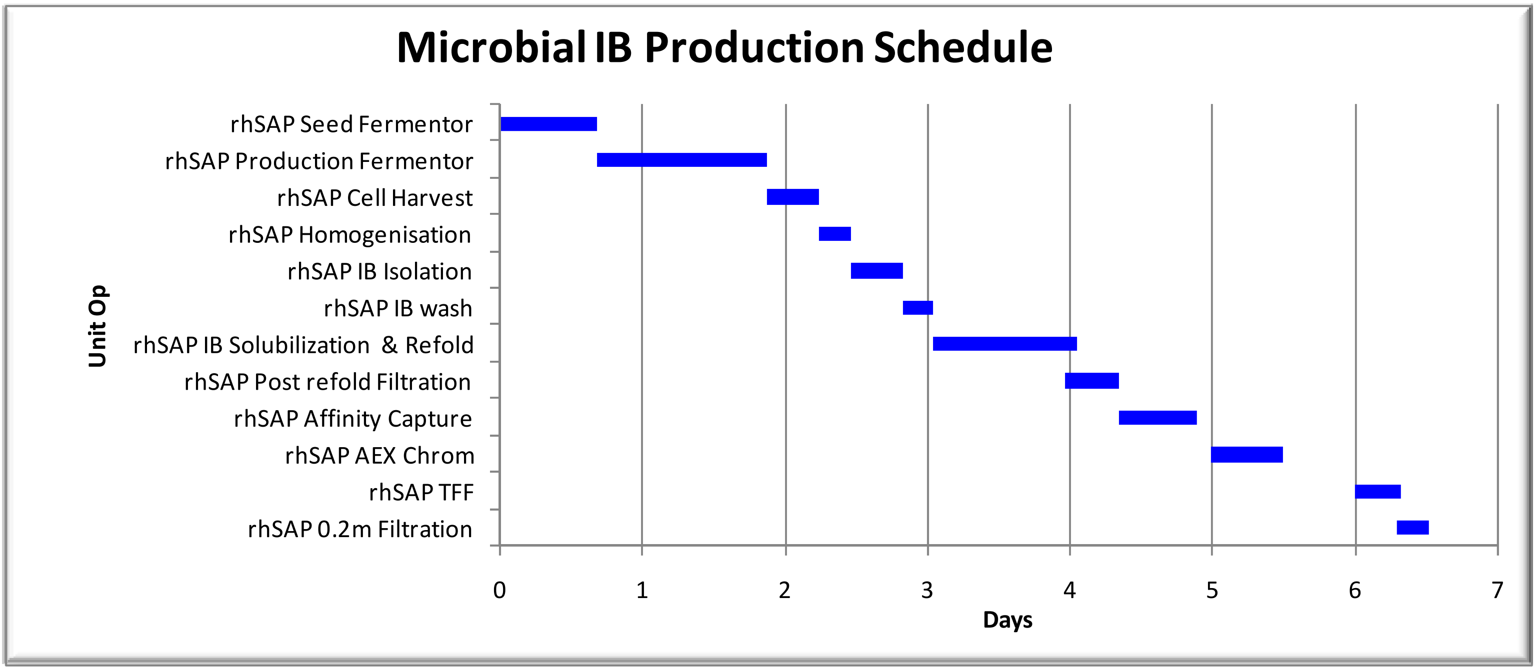
Production Schedules: CHO Process

- Model output includes production schedules to allow assessment of processing bottlenecks and annual throughput



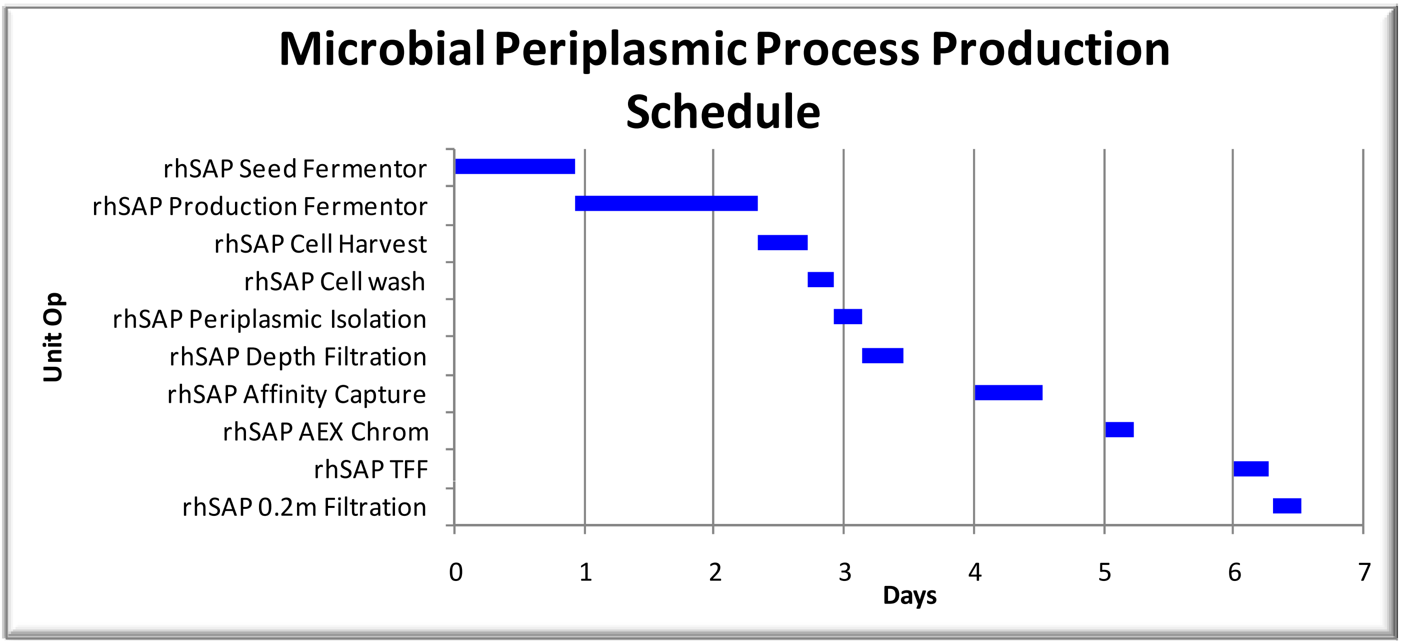
- 200 L CHO Process requires 404 h processing time (not including holds)
  - Appx 21 plant days at 200 L scale
  - Cell culture is 90% of processing time
  - 34 Batches/year
  - 429 hours at 10,000 L scale

Production Schedules: Microbial Refold



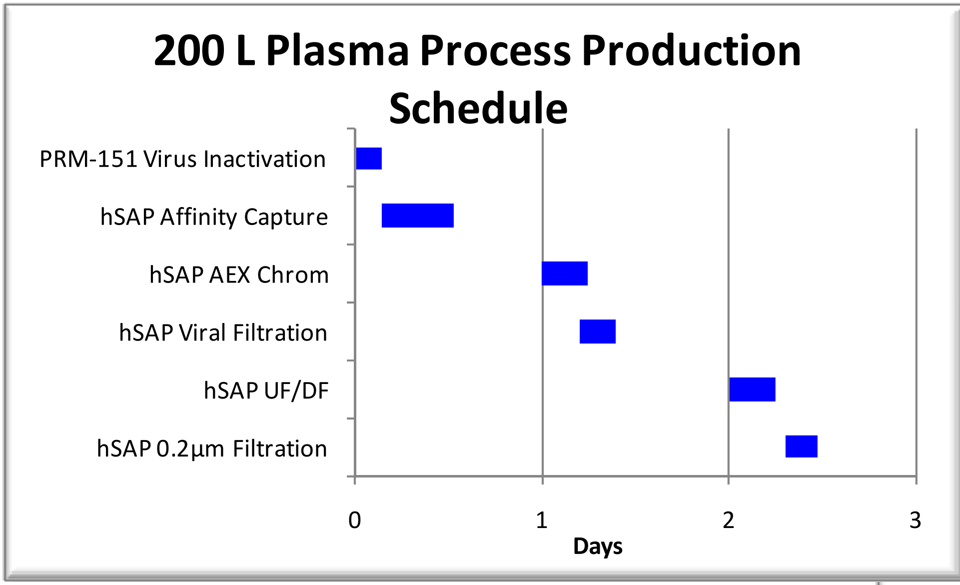
- 200 L Microbial Refold Process requires 136 h processing time
  - Appx 7 plant days at 200 L scale
  - Fermentation is 33% of processing time
  - 209 Batches/year
  - 159 hours at 10,000 L scale

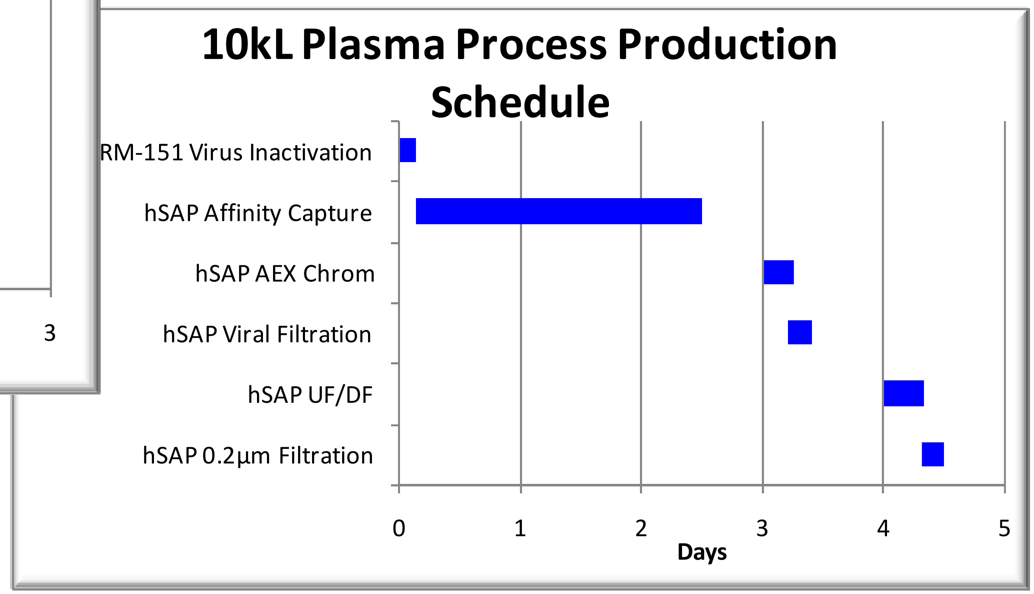
Production Schedules: Microbial Periplasmic Process



- 200 L Microbial Periplasmic Process requires 112 h processing time
  - Appx 7 plant days at 200 L scale
  - Fermentation is 50% of processing time
  - 209 Batches/year
  - 130 hours at 10,000 L scale

Production Schedules: Plasma Process





- 200 L Plasma Process requires 33h processing time
  - Appx 3 plant days @ 200 L scale, 5 days @ 10kL scale
  - Major block of process time is loading capture column
    - 28% at 200 L, 70% at 10 kL
  - 423 Batches/year @ 200 L, 105 Batches/year @ 10kL
  - 83 hours at 10,000 L scale

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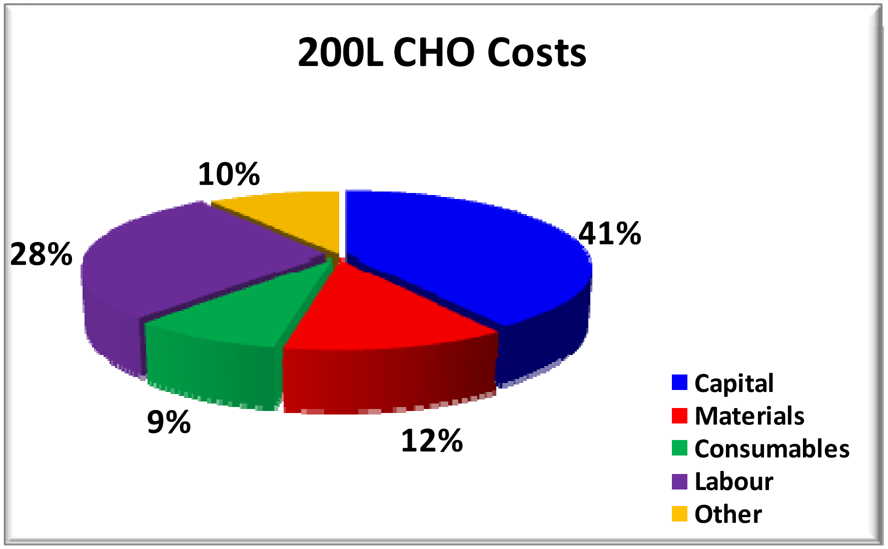
Process COGs Analysis Overview

Cost Categories & Detail

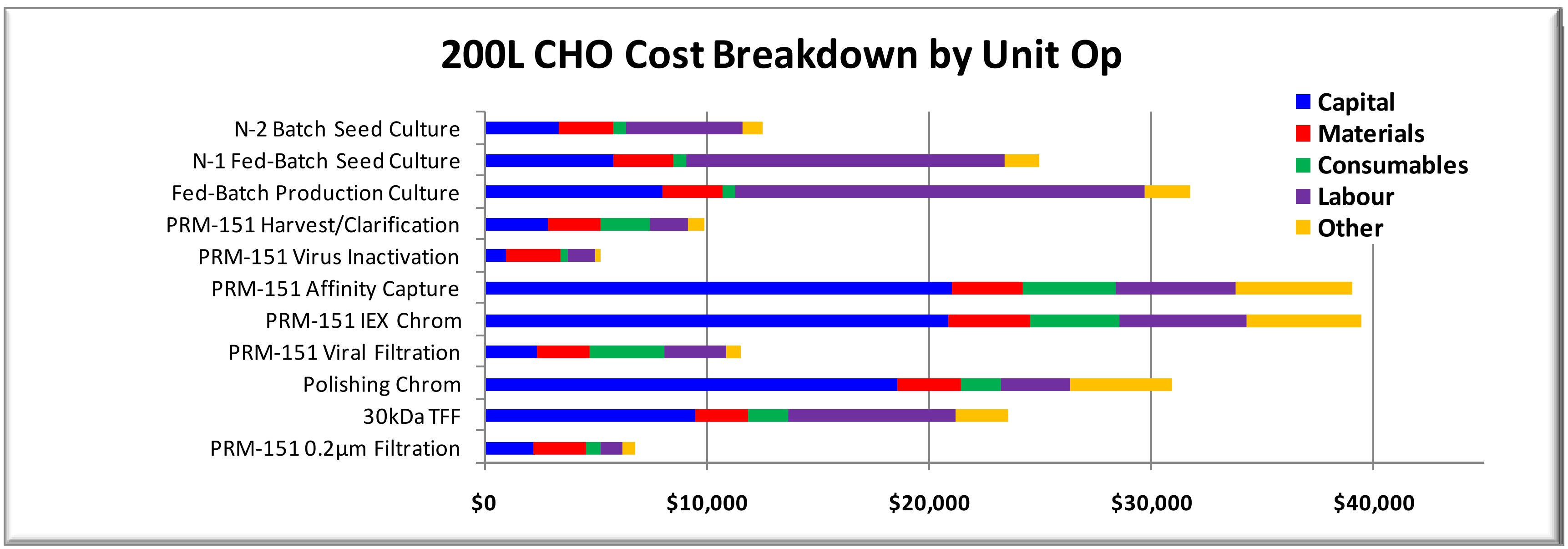
1	Capital	Capital Charge
2	Materials	Media
		Buffer
		Bought WFI
		CIP
		QC tests
3	Consumables	Resins/MA
		Bags
		Filters
4	Labour	Process
		Quality
		Indirect
5	Other	Insurance & other
		Waste management
		Maintenance
		Utilities

- Costs are broken down into 5 categories
  - Capital
  - Materials
  - Consumables
  - Labour
  - Other
- Costs are also broken down by unit op
- Process scenario analysis of base case
  - ± Disposables
  - at 200 L and 10,000 L scales
- Process sensitivity analysis
  - ± Disposables (red = SS, blue = Disp)
  - Scale
  - Product titer
  - # Bioreactors
  - % Capacity Utilization

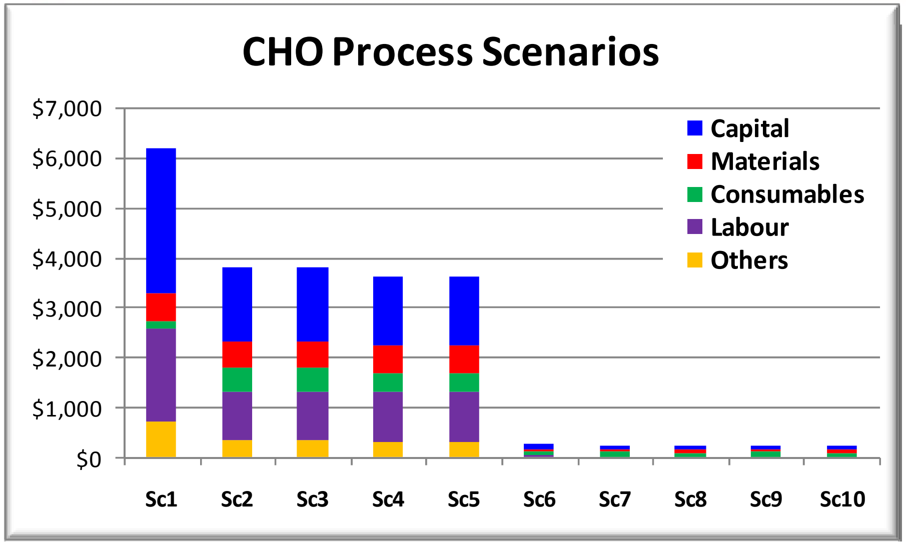
CHO Process COGs Analysis



- $4,321/gram
- $225,000/batch
- Largest category is capital @ 41%
- COGs fairly well balanced across category and unit op



CHO Process Scenarios

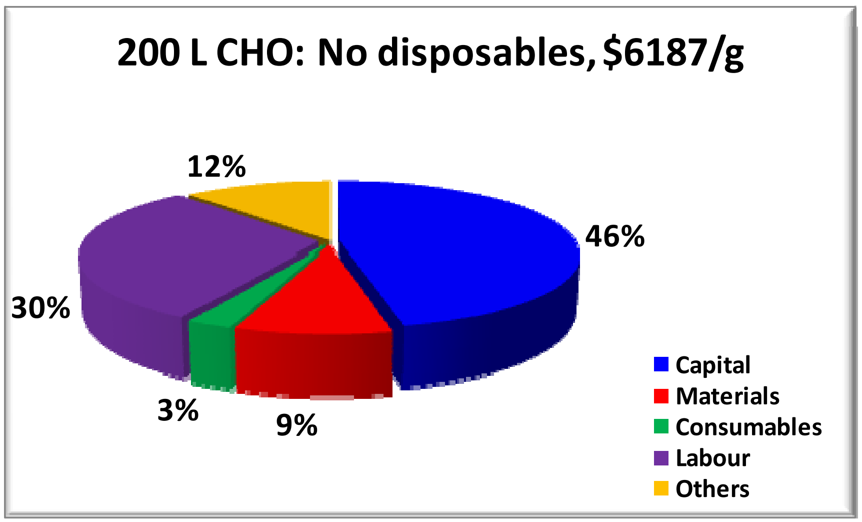


For all scenario analyses:

- 1-5 are 200 L
- 6-10 are 10,000 L
- 1 & 6 are no disposables
- 2-5 & 7-10 are disposable variations

- + Disposables reduces $/gram
- Biggest $/g reduction factor is scale

Worst



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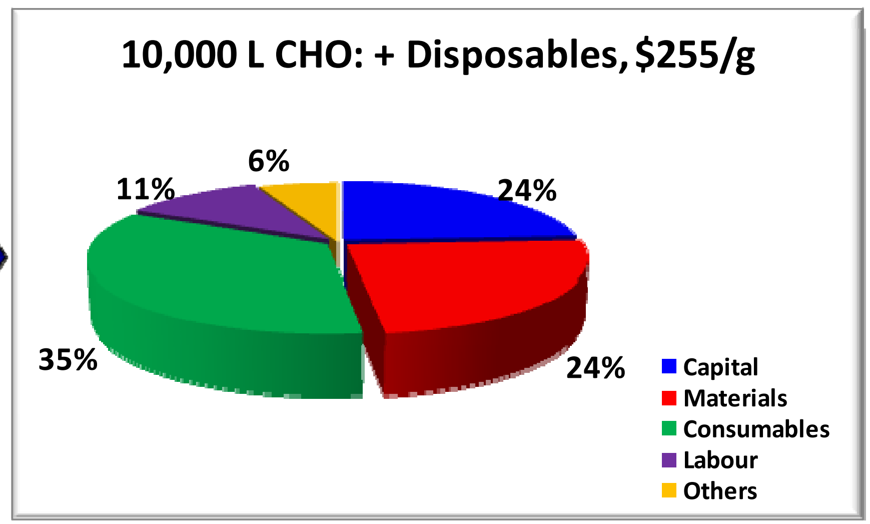

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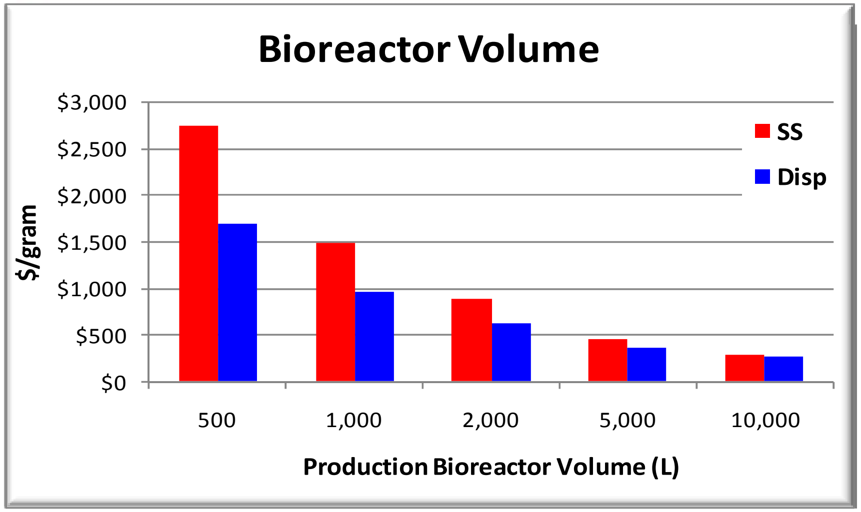
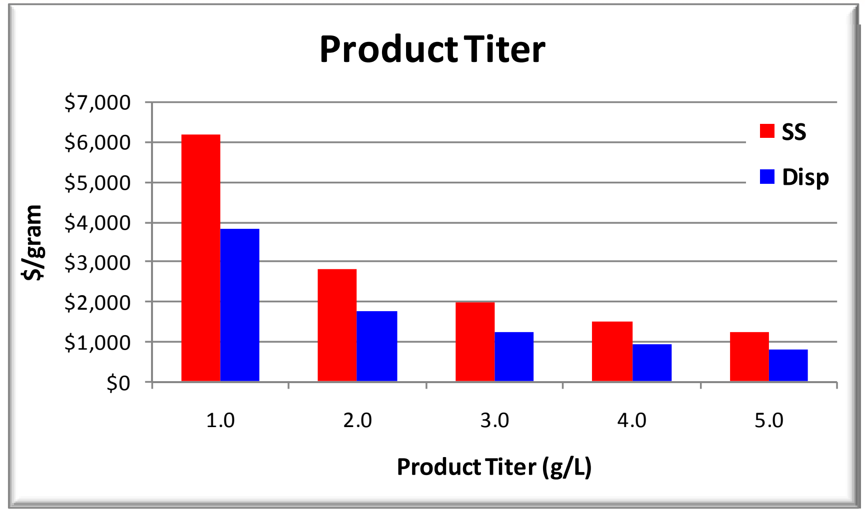
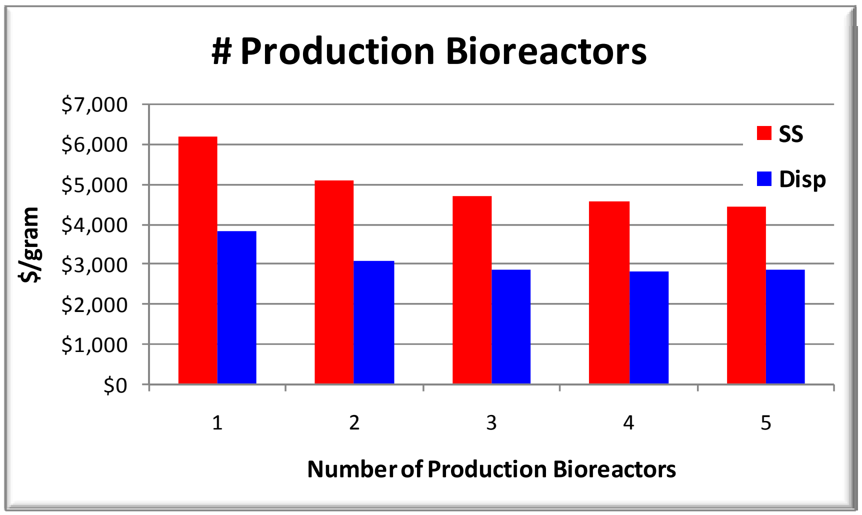
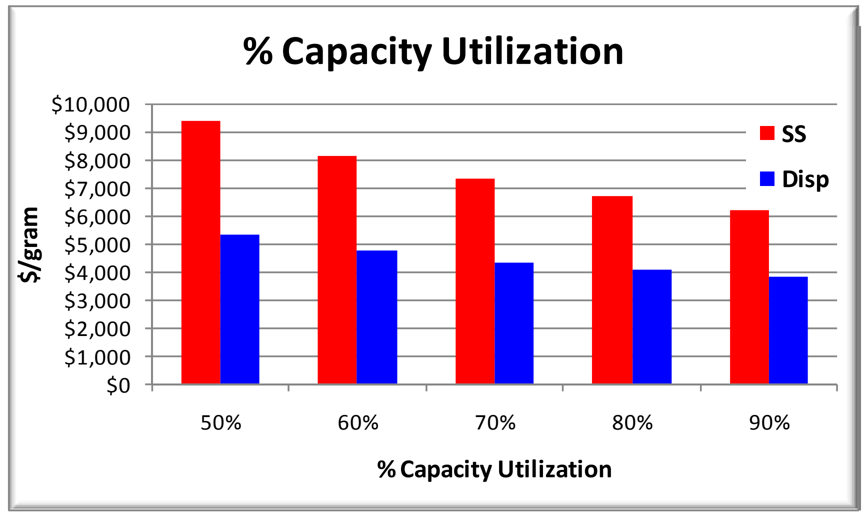
CHO Process Sensitivity ± Disposables









- Most sensitive to bioreactor volume and product titer
- Disposables have greatest benefit at smaller scale

Microbial IB Process COGs Analysis

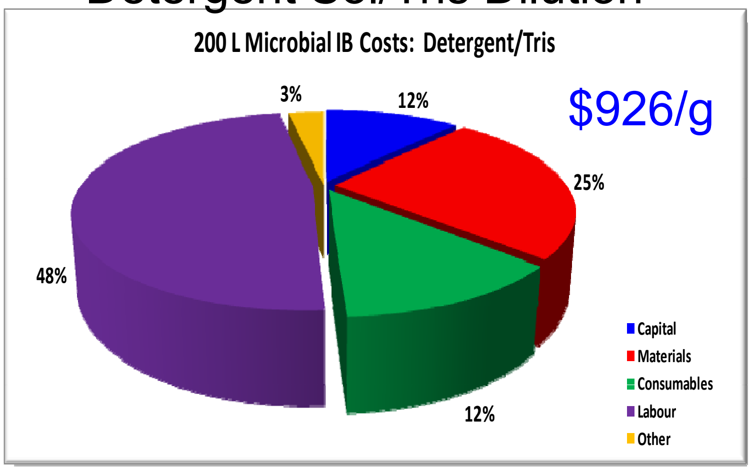
Compared two refold process alternatives at 200 L and 10,000 L scale:

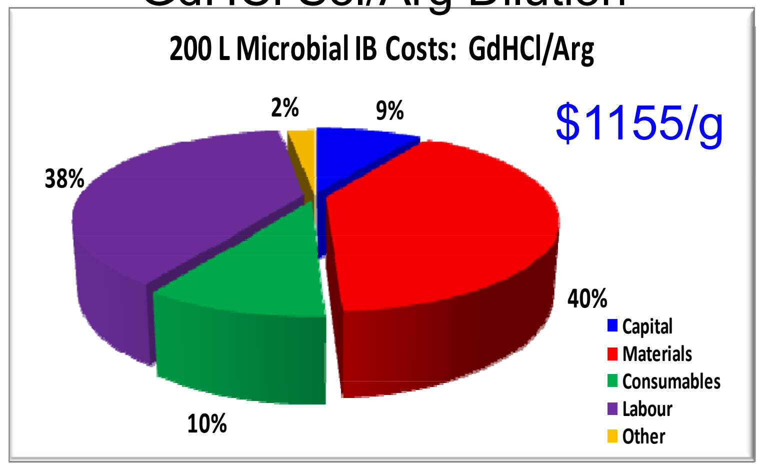
1. Solubilize in detergent, dilute into Tris refold buffer
2. Solubilize in GdHCl, dilute 10x into 0.5 M Arginine refold buffer

Detergent Sol/Tris Dilution

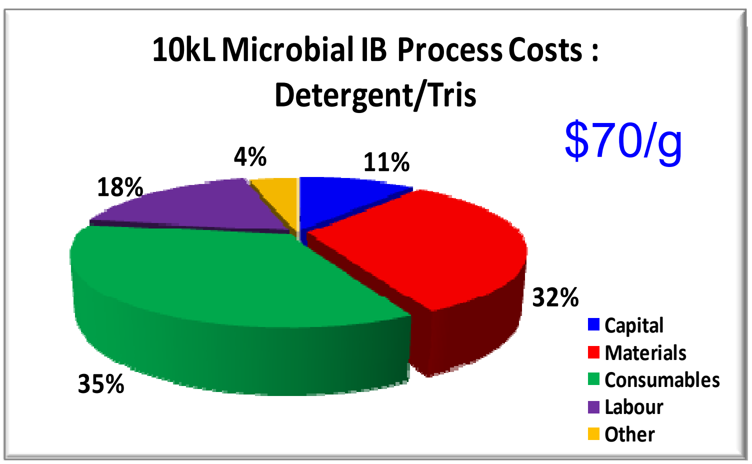
GdHCl Sol/Arg Dilution

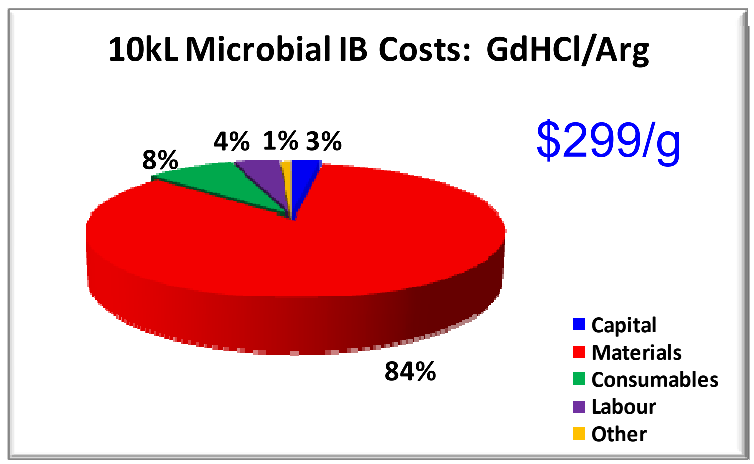
200 L





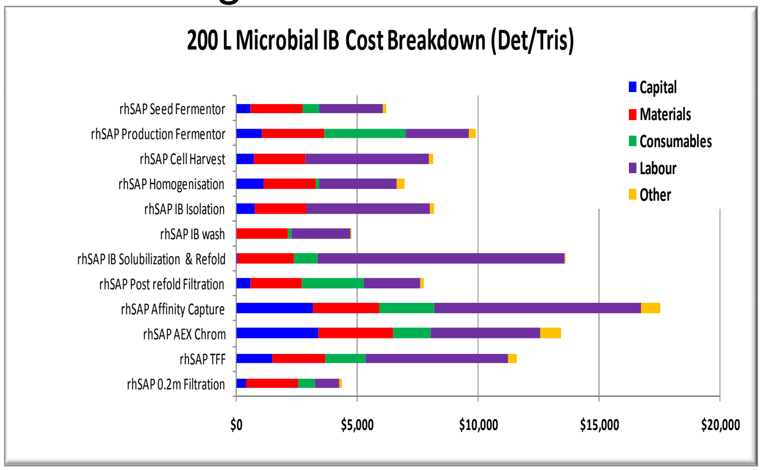
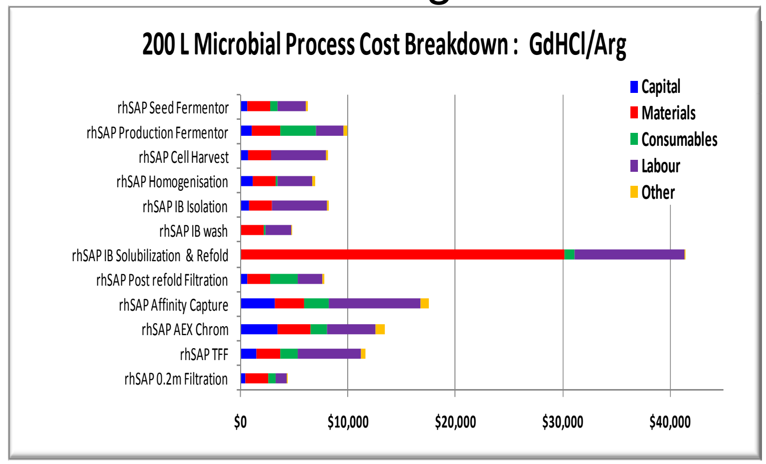
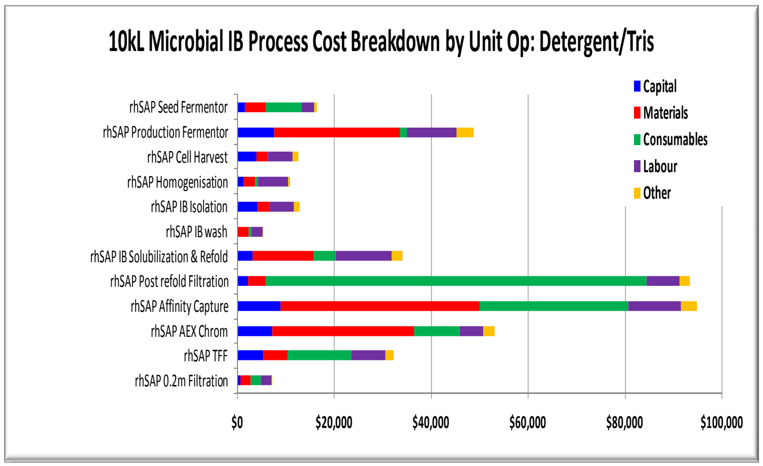
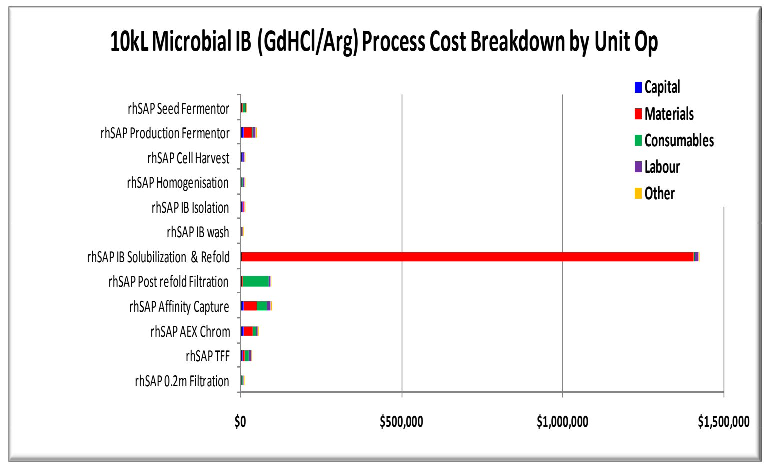
10 kL





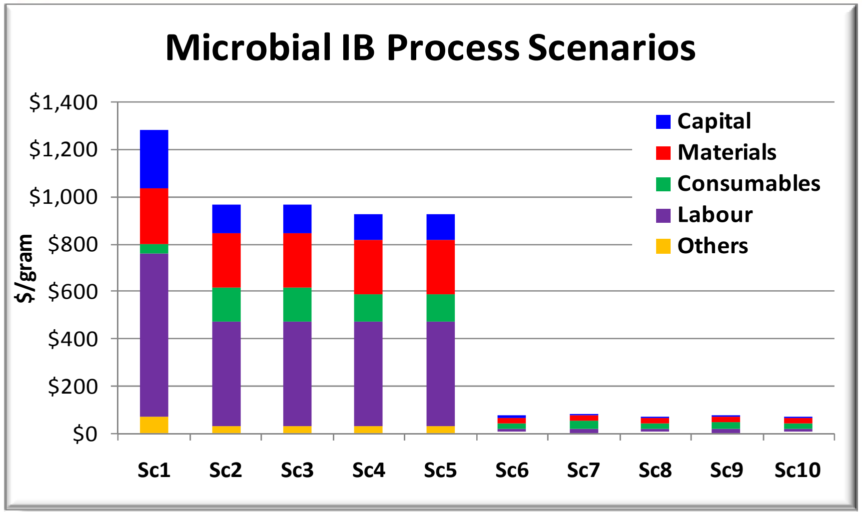
- Choice of refold system dramatically affects COGs

Microbial IB Process COGs Breakdown by Unit Op

	Detergent Sol/Tris Dilution	GdHCl Sol/Arg Dilution
200 L		
10 kL		

- Quick visual read of where the major costs are and how they're balanced
- Try to avoid GdHCl/Arg, or at least minimize [Arginine]

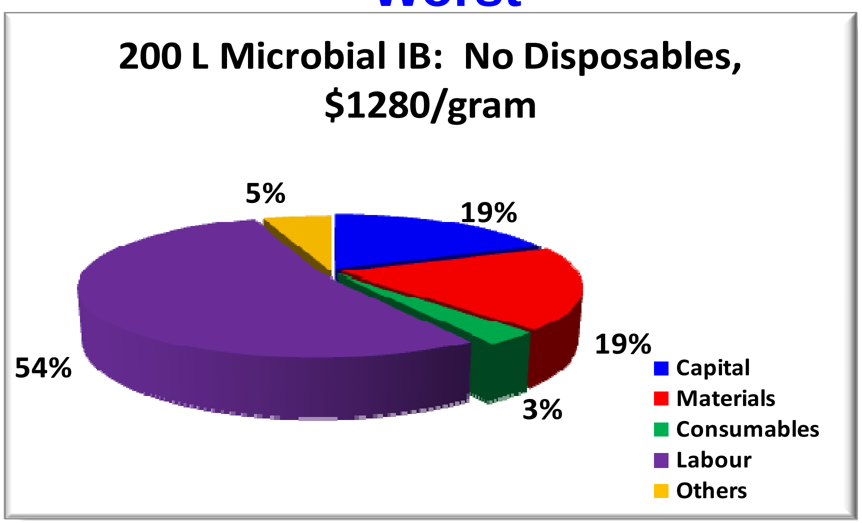
Microbial IB Process Scenarios (Detergent/Tris Refold)



As before:

- + Disposables reduces $/g
- Biggest $/g reduction factor is scale
- Upon scaleup from 200 to 10,000 L
    - Labor % decreases 3x
    - Materials % increases 10x
- Similar trends for GdHCl/Arg refold

Worst



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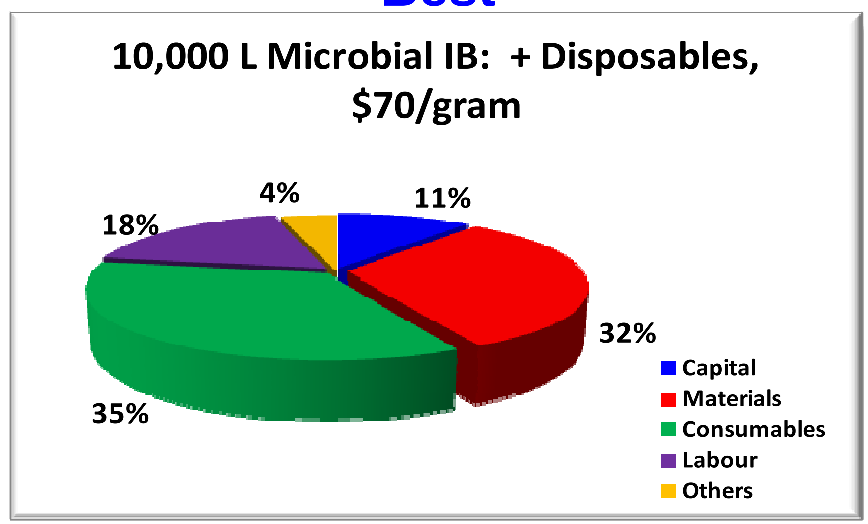
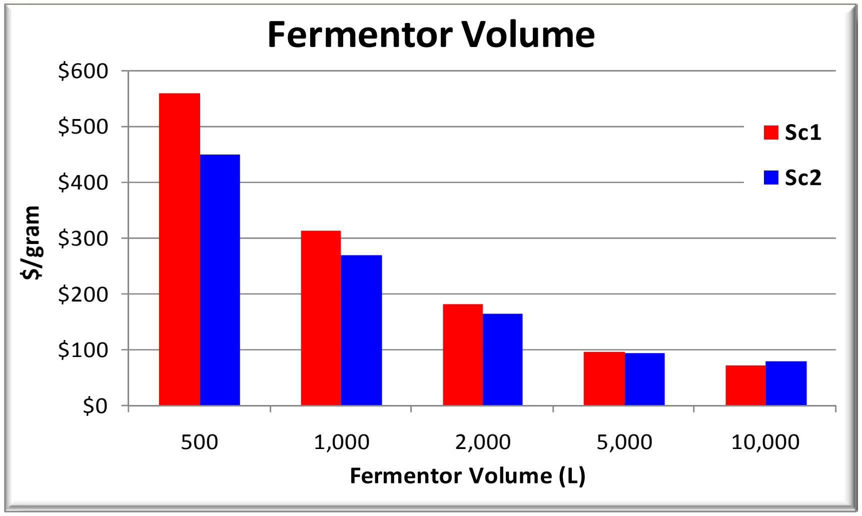
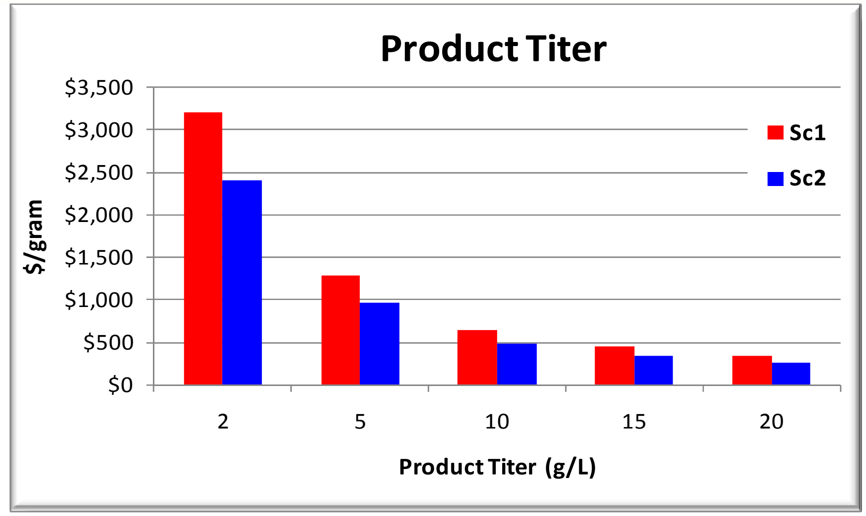
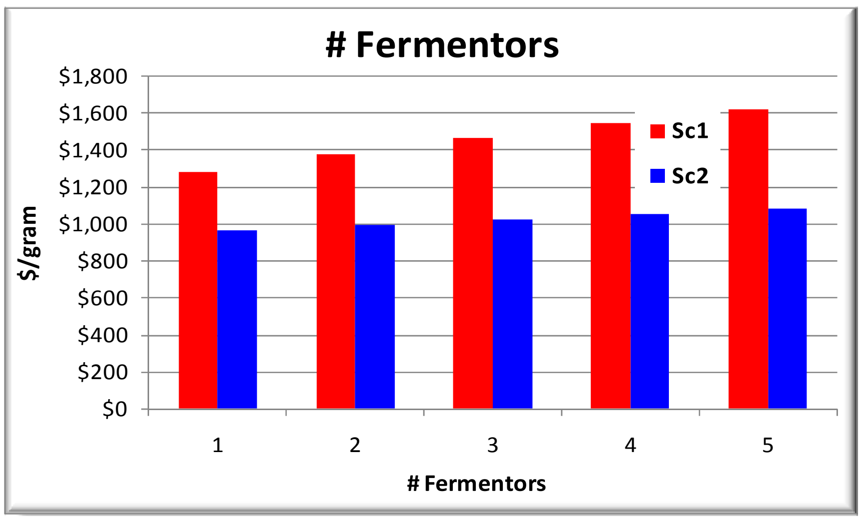
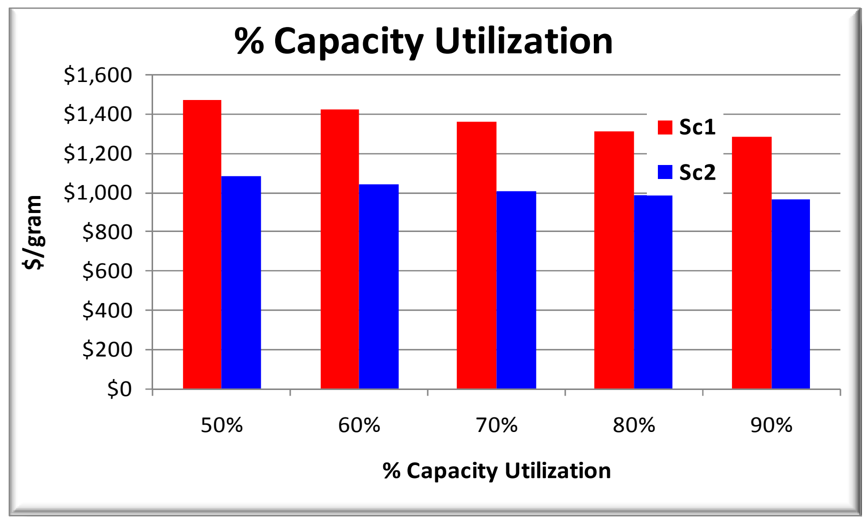


Microbial IB Process Sensitivity ± Disposables









- Fewer fermentors better
- Similar trends for GdHCl/Arg refold process

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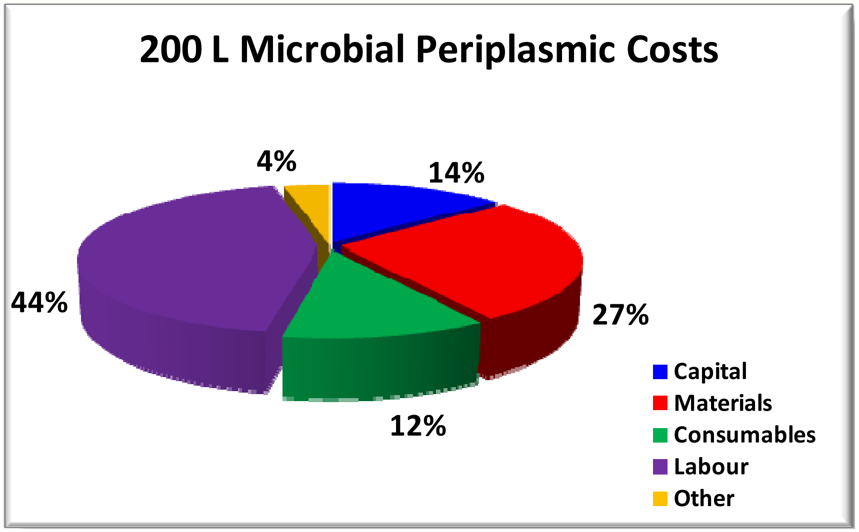
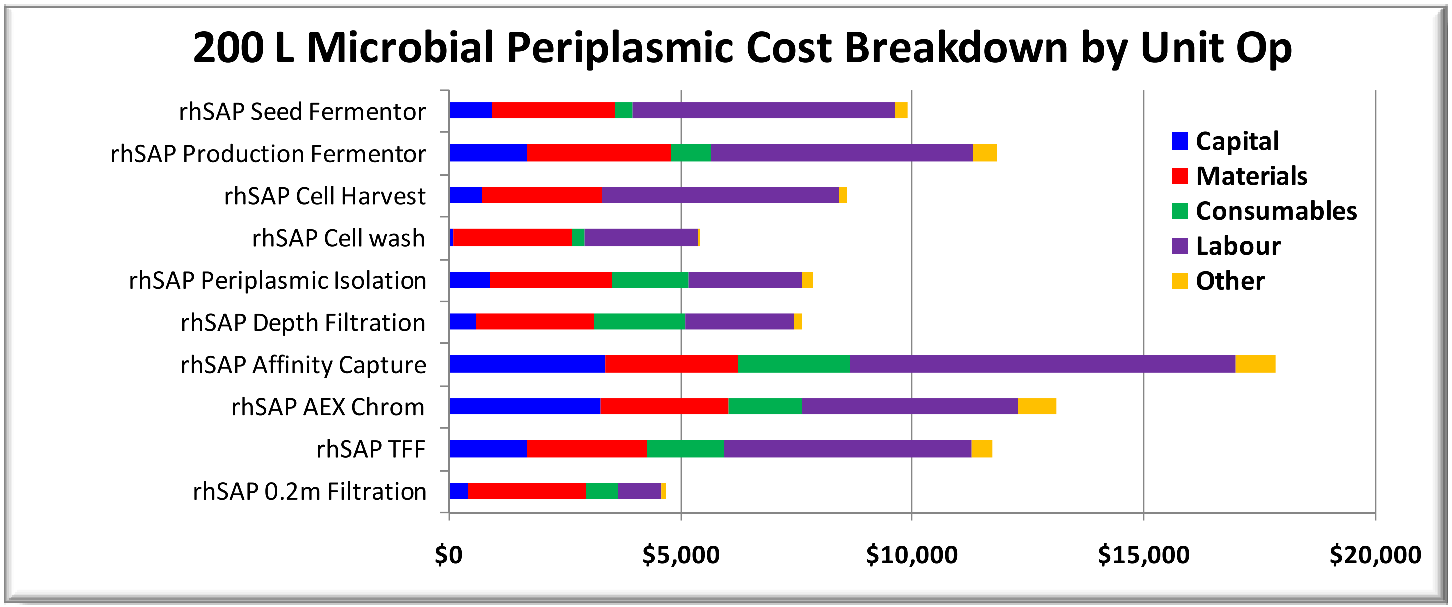
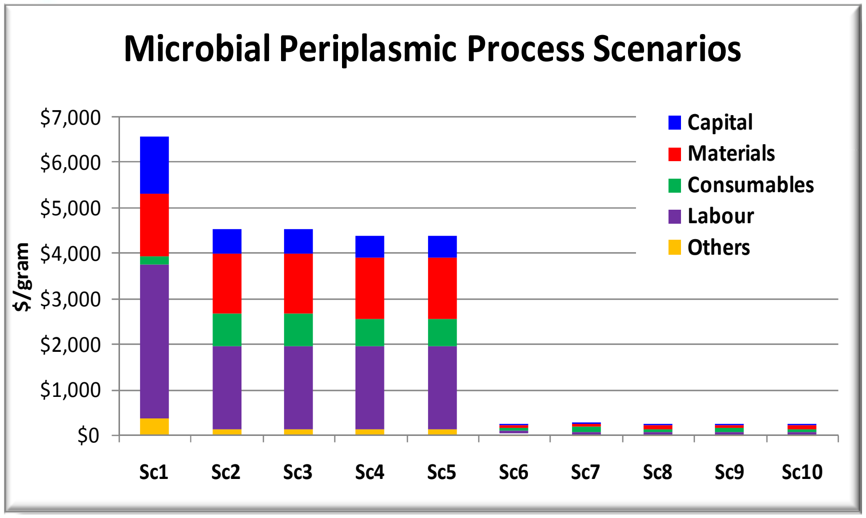
Microbial Periplasmic Process COGs Analysis



- $4,904/gram
- $98,500/batch
- At small scale, largest category is labour @ 44%
- Costs fairly balanced across unit op

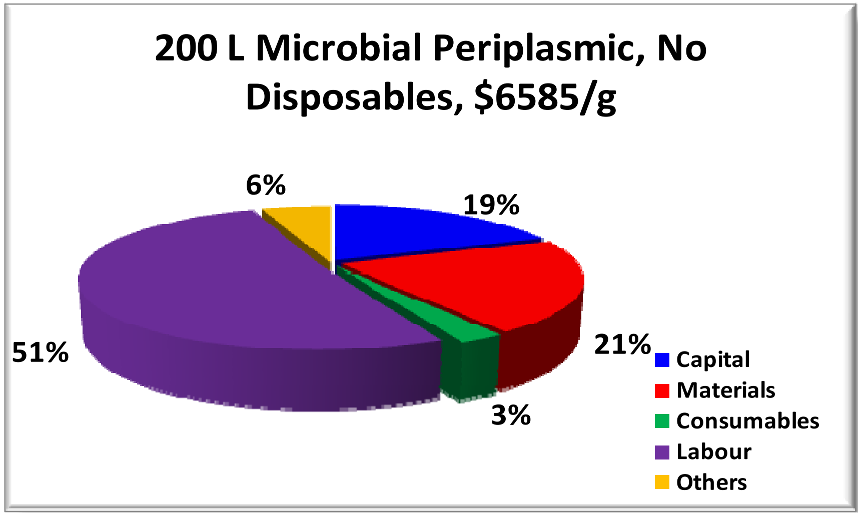


Microbial Periplasmic Process Scenarios



- Labour costs dominate at small scale
- Labour, Materials, Consumables balance at large scale
- Low expression level (0.25 g/L) results in high cost at small scale

Worst



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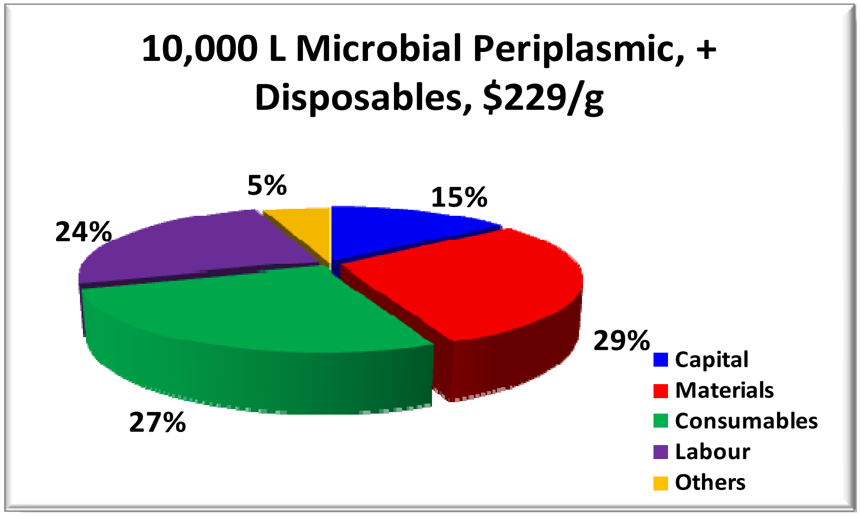
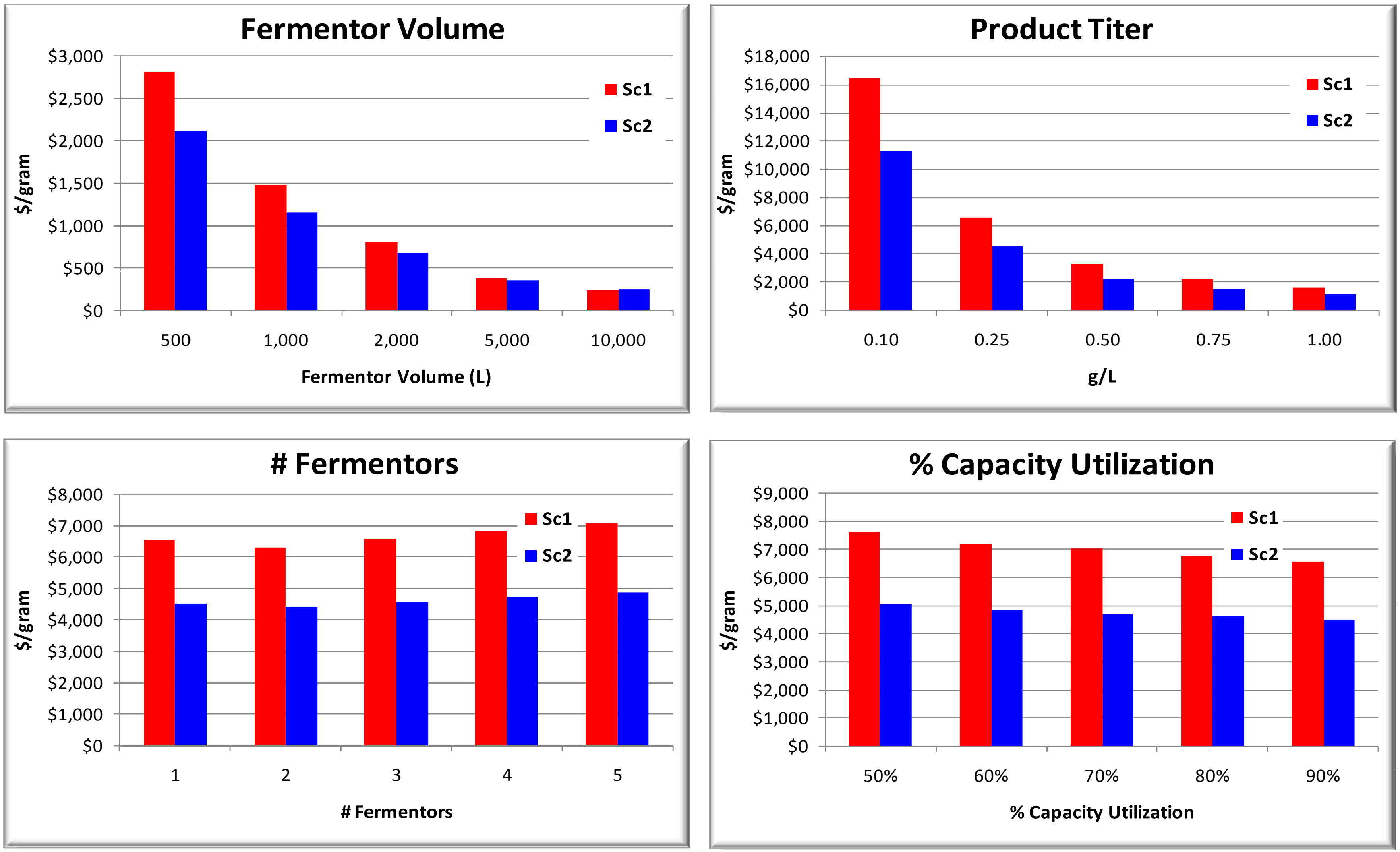
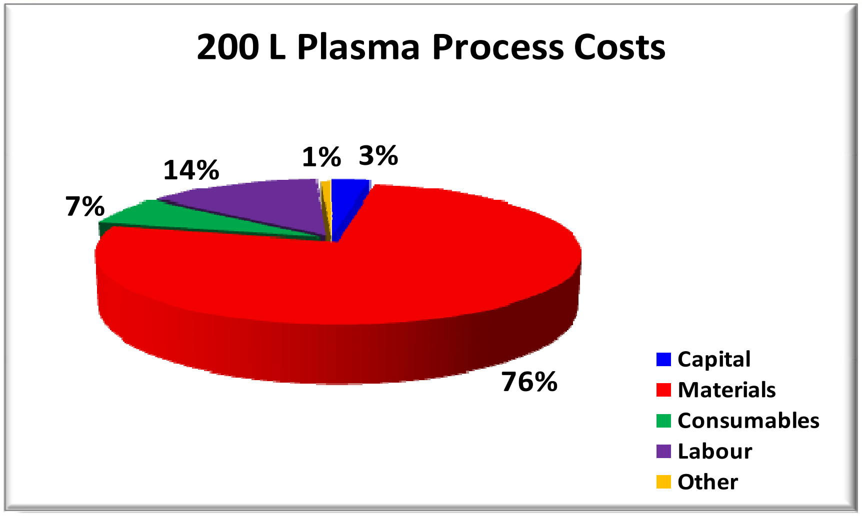


Microbial Periplasmic Process Sensitivity ± Disposables

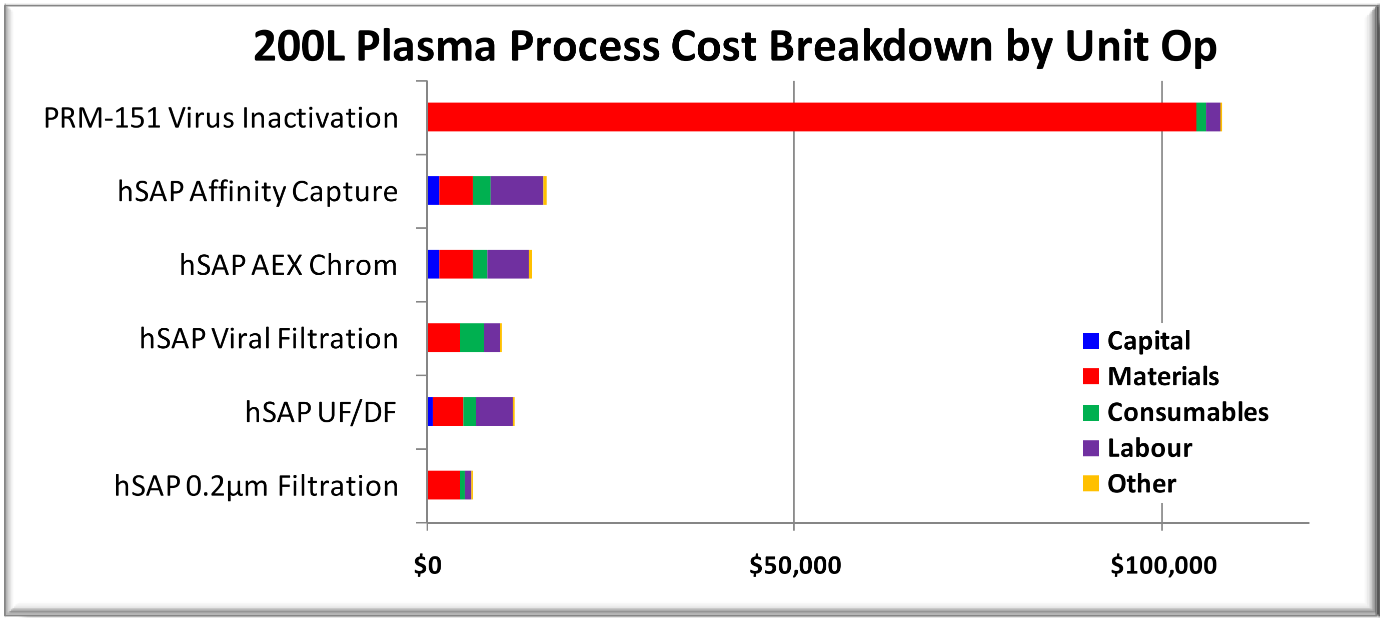


- Similar trends as for refold process

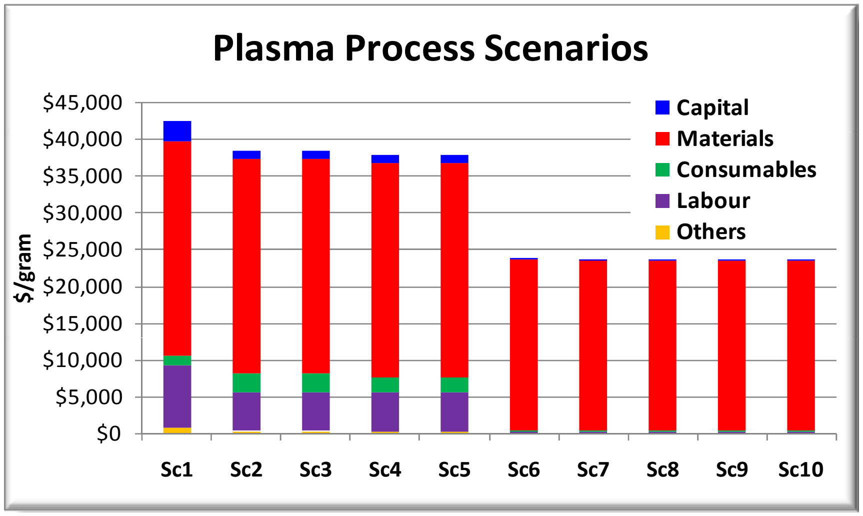
Plasma Process COGs Analysis



- $38,371/gram
- $166,742/batch
- Cost entirely dominated by price of human plasma (> $500/L)
- Virus inactivation step includes cost of feedstock

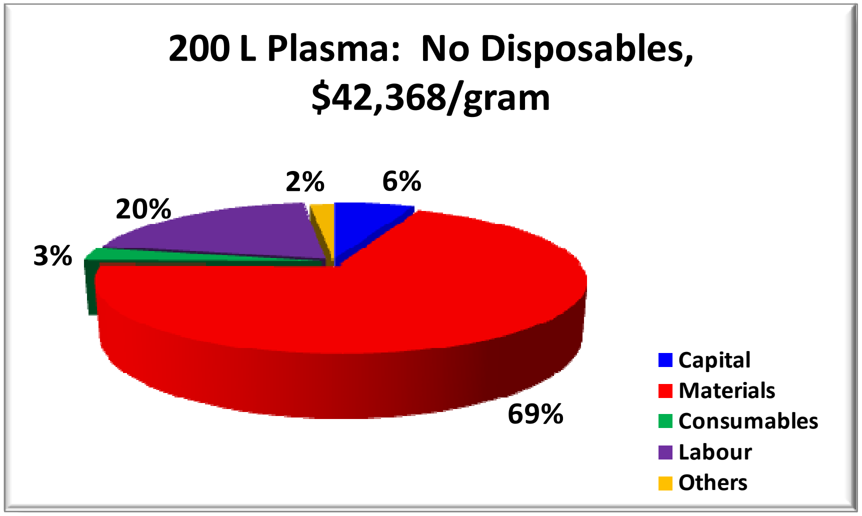


Plasma Process Scenarios

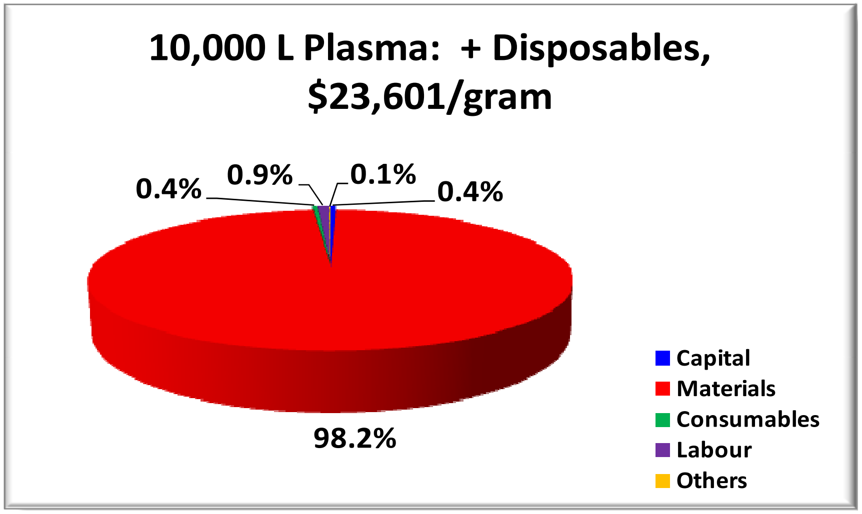


- Very low abundance (0.03 g/L) and high cost of human plasma make this uncompetitive with recombinant sources
- Plasma is difficult to source
- ± Disposables makes little affect
- No scale advantage

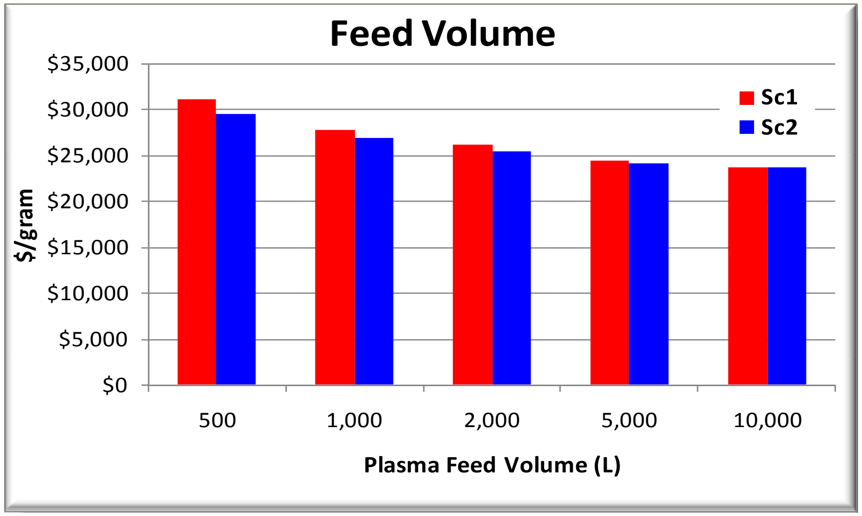
Worst

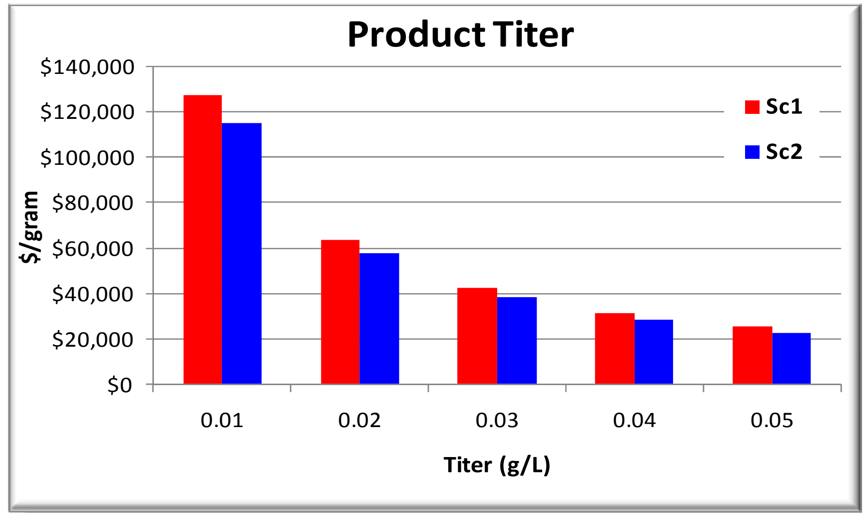


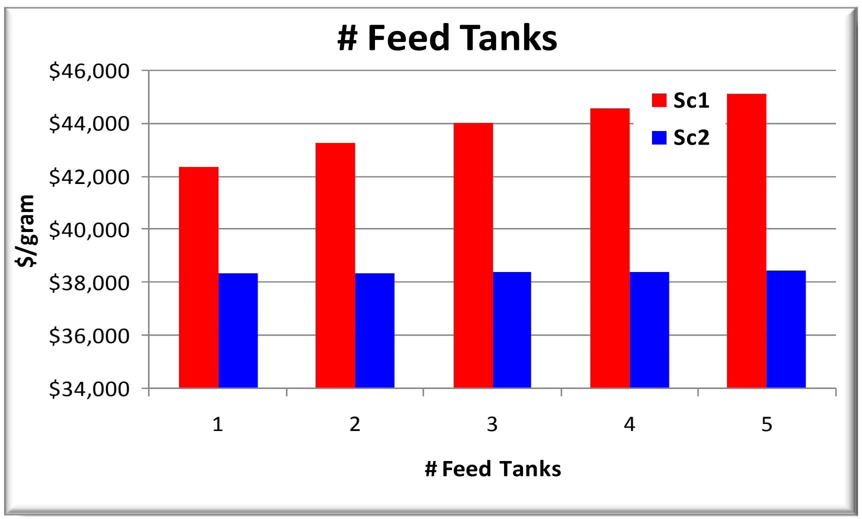
Still Bad

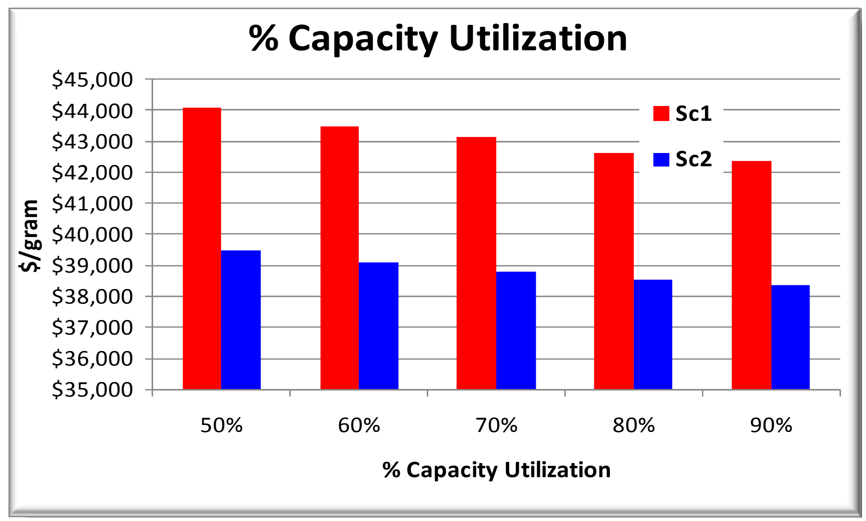


Plasma Process Sensitivities



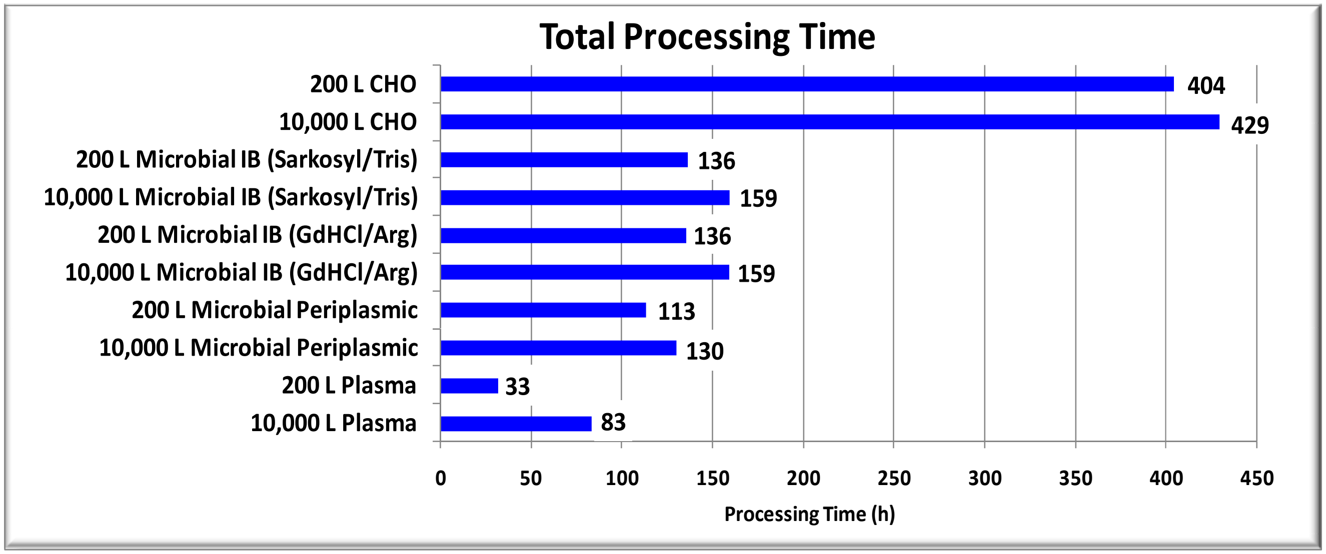


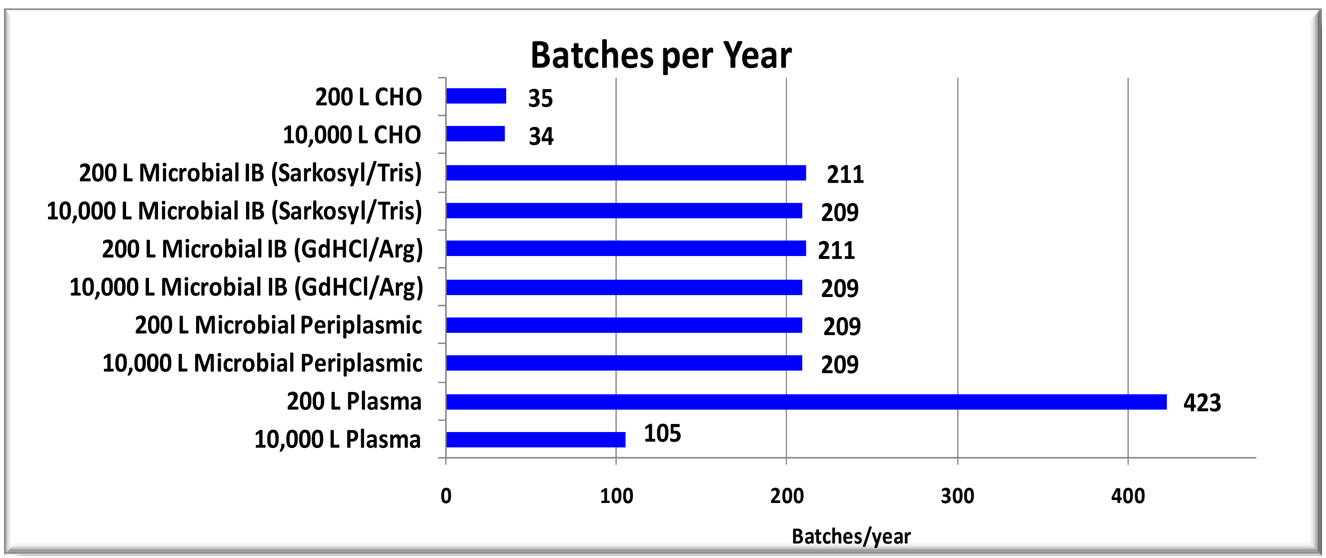




- Not very sensitive to scale
- Increase in titer above 0.03 g/L unlikely

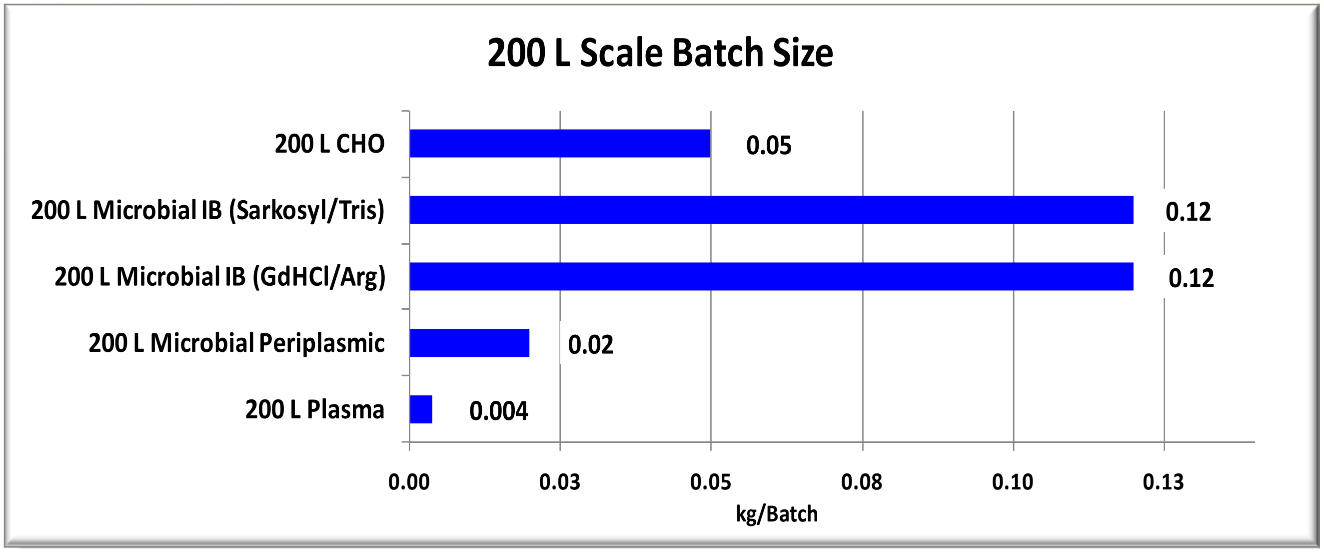
Process Comparisons: Processing Time & Batches/Year

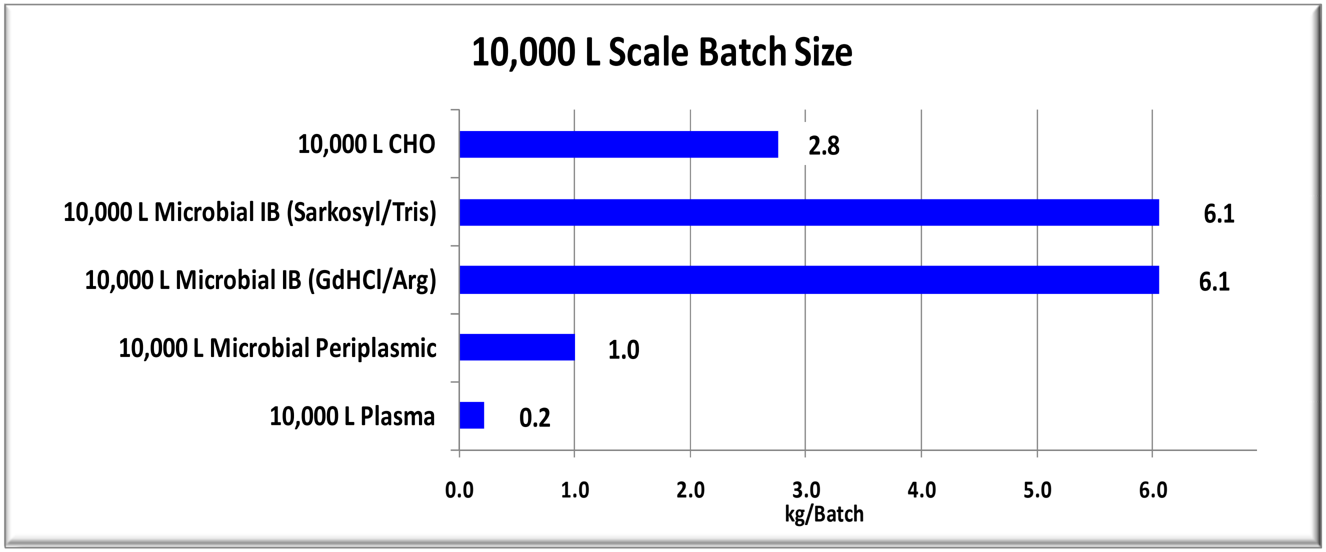




- Process sequence determines turnaround time and batches/year
- CHO process bottleneck is cell culture

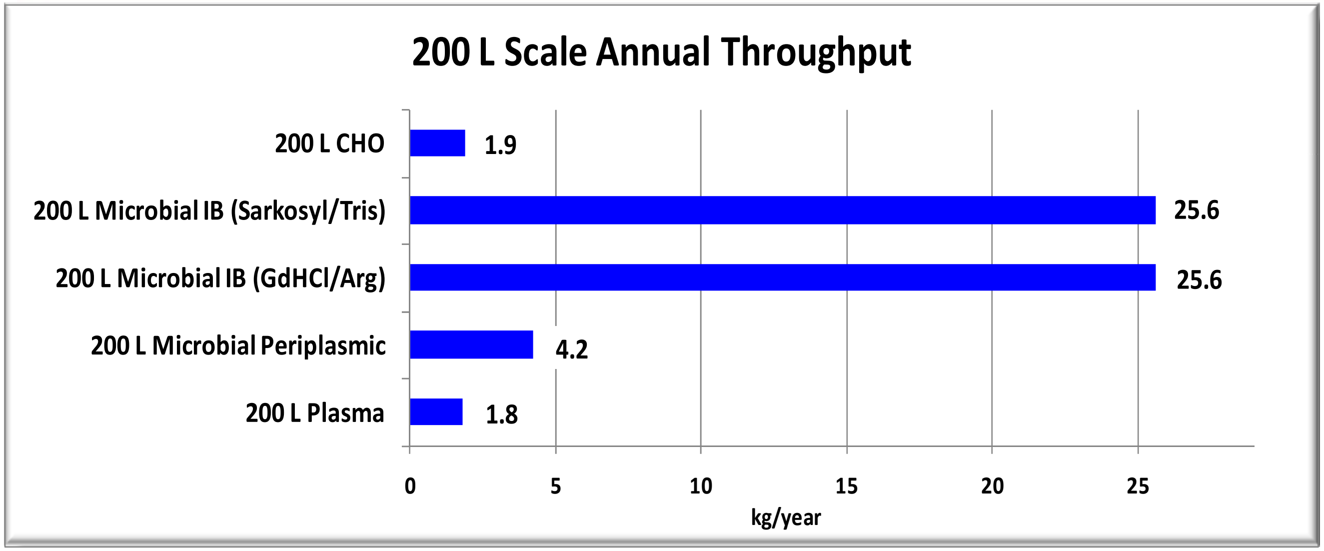
Process Comparisons: Batch Size at 200 & 10,000 L

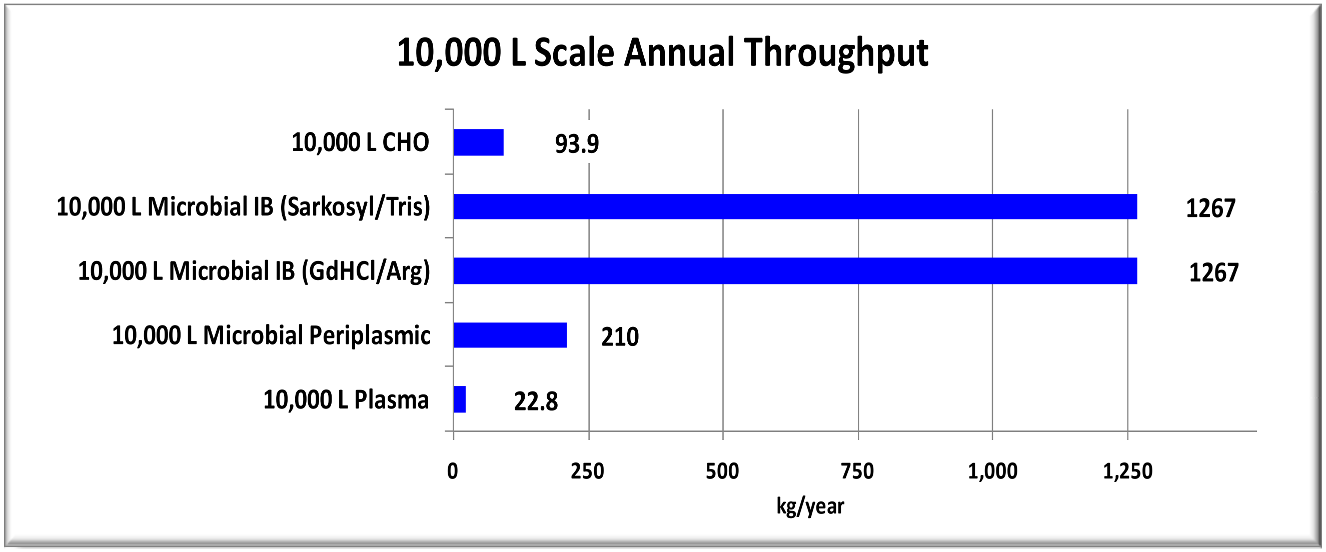




- Expression levels drive batch sizes more than process yields
- Comparing batch sizes helps with production planning
- Batches below 50 grams lose too much to release and stability testing

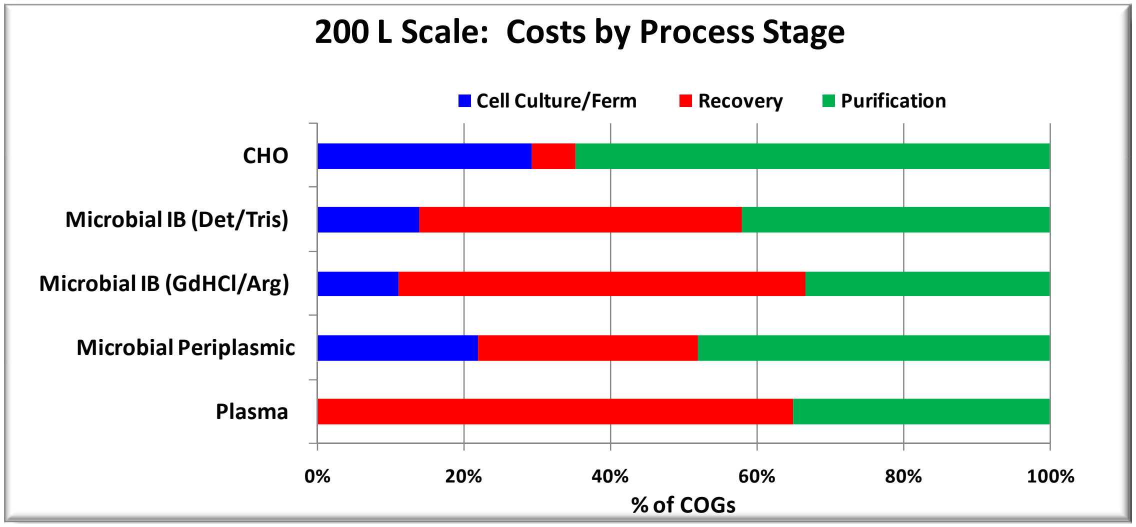
Process Comparisons: Annual Throughput

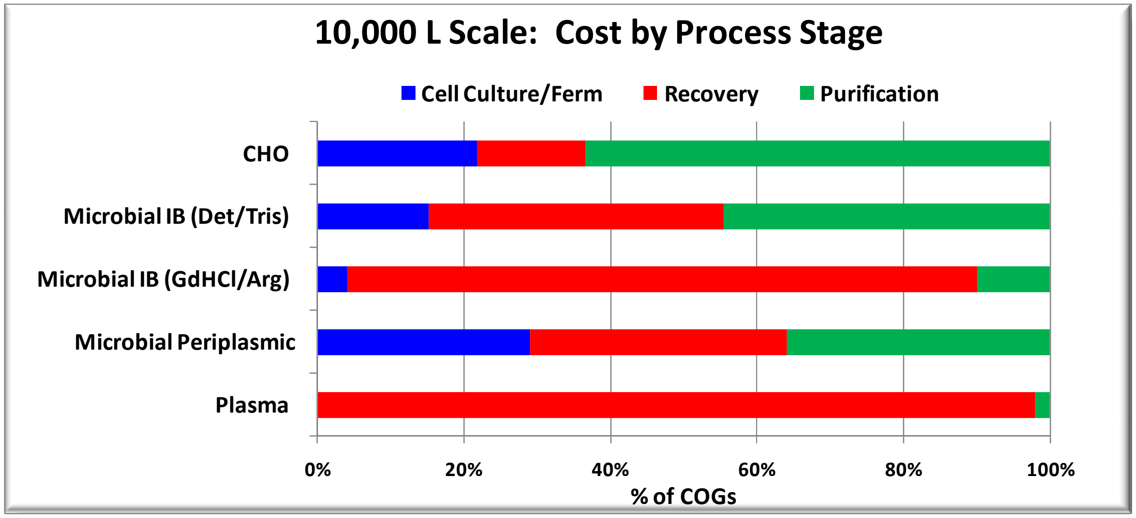




- Comparison of annual throughput guides expression system preference and scale to meet projected demand

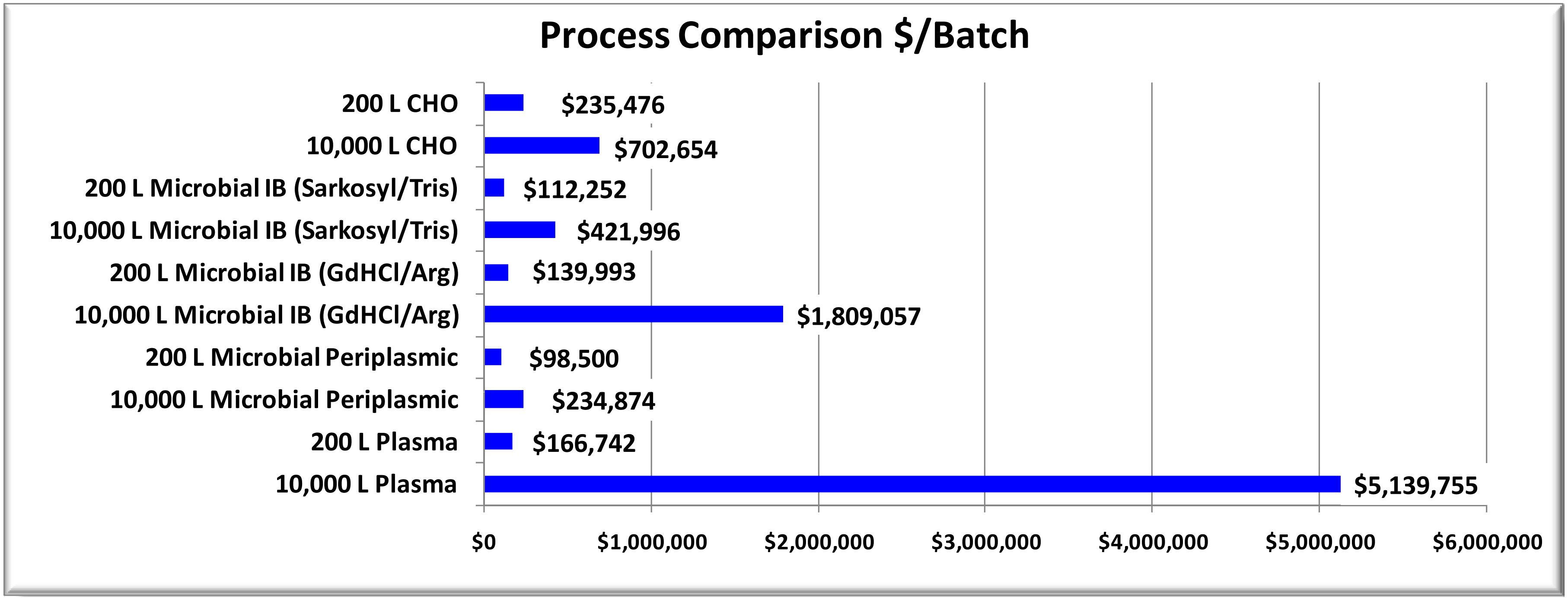
Process Comparisons: Costs by Process Stage





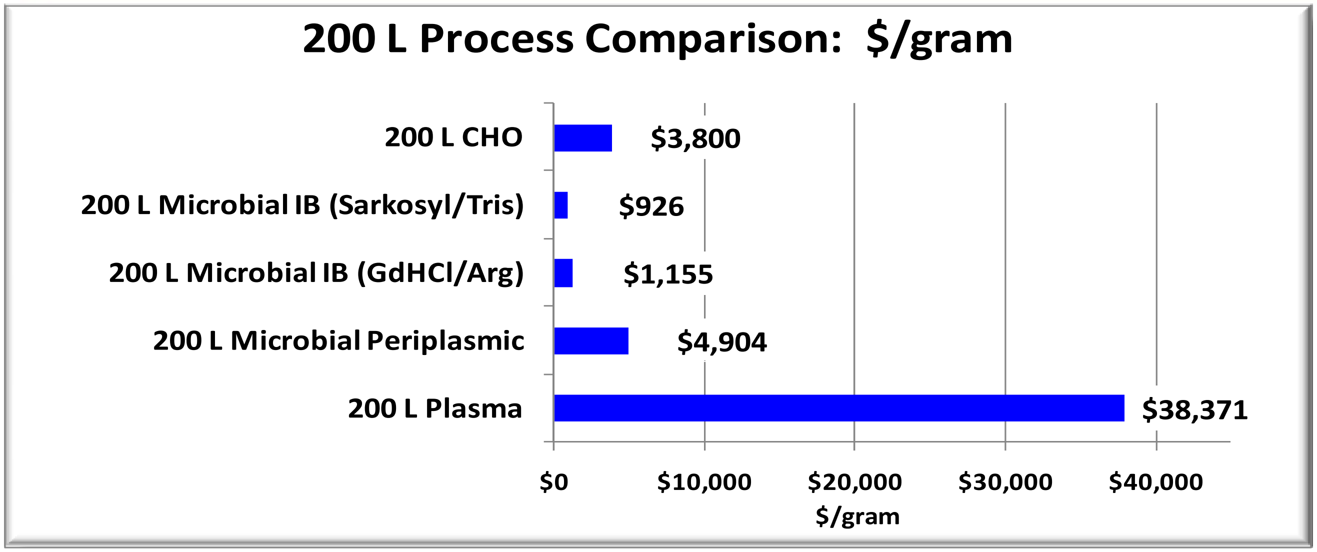
- Process stages are:
  - Cell Culture
  - Recovery
  - Purification
- Provides coarse view of cost allocation
- Helps focus PD effort

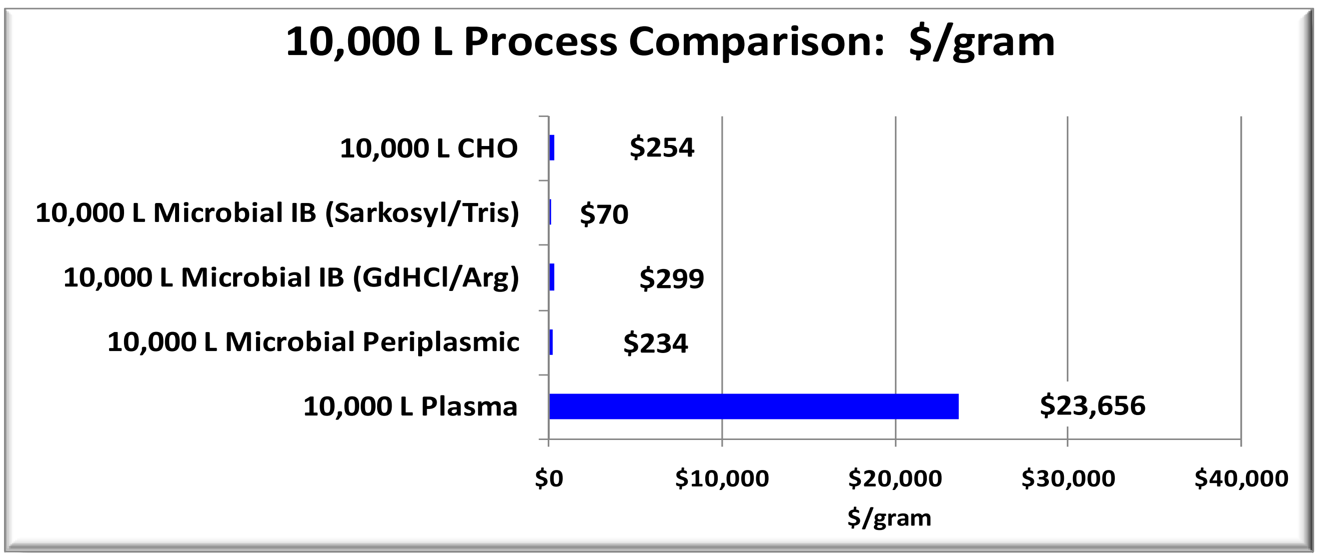
Process Comparisons: Cost per Batch



- Microbial processes are generally less costly per batch
- GdHCl/Arg refold process is materials intensive at large scale
- Large scale plasma process is cost prohibitive

Process Comparisons: $/Gram





- Process Alternatives carry 3 orders of magnitude range in COGs
- Sourcing from plasma is a clear loser
- Refolding from E. coli most economical IF you don't need GdHCl/Arg system

PROMEDIOR

Conclusions

- Cost modeling early in development is a useful project management tool
  - Allows detailed and flexible look at production cost drivers
  - Facilitates expression system/process design choices
  - Focuses process development effort
  - Frames discussion for longer-range planning
- As expected, increasing product titers has major benefit to COGs
  - Take time to develop appropriate cell line
  - Work hard early to maximize expression levels
  - Understand future limitations as function of expression level
- Increasing scale also reduces COGs
  - Assessing costs at scale can highlight problems to address early