QUALITY THROUGHOUT THE PRODUCT LIFECYCLE

Bioprocessing Challenges: High-Titer Mammalian-Based Cell Systems

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Biogen

Business Drivers

- Elements influencing the way Biologics may be manufactured /supplied in the future

Cost of Raw materials Advent of Biosimilars Reimbursement pressures	Squeeze in Profit Margins	Drives Reduction in Costs
Advent of Enhanced Cell line screening & Productivity	Process /Facility Bottlenecking	Drive to Debottleneck and Compress entire Process Train
Expanding & Emerging Products Market	Supply Reliability	Drive Process, Facility and Supply Flexibility

Cost Impacts

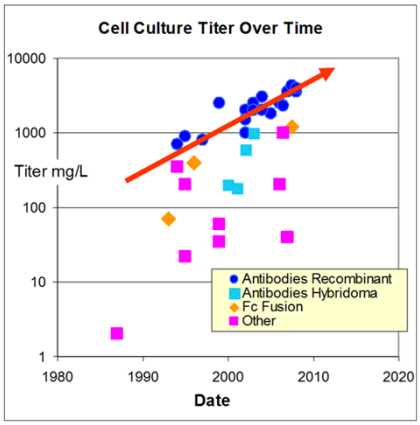
Profit Margins for new and existing drugs will be squeezed

- Increasing Costs of Operations, Raw materials
- Competition between Innovator and Biosimilar products
- Drive for reduction in Healthcare costs
  - Reimbursement pressures
  - Consolidation of Health providers

Mandates for Bio manufacturing Operational Changes

- Reduce Cost per unit mass of product produced

Future state of Biologics processing



** Data from Thomas Ryll, IBC 2009*

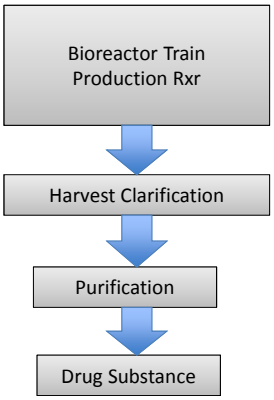
Current/Future state of Cell Culture:

- technology has evolved and high titer processes (>5 g/L) are norm

Current state of biologics process:

- Increased time in Production Reactor
- bottlenecks in processing at >5 g/L
- buffer volumes too large
- excessive column cycling
- column capacity exceeded
- filtration Areas & Processing time Increase

Impact of Production Reactor titer on Throughput



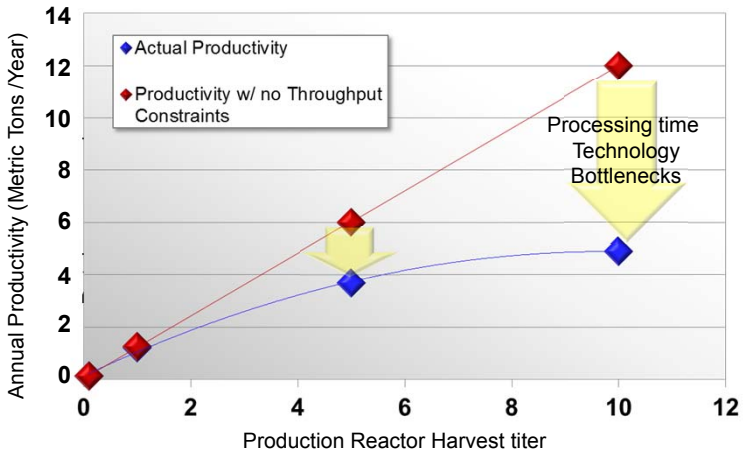
Production Scale

Production reactor Titer (g/L)	15K Liters: Amount Produced annually (Kg)	2K Liters: Amount Produced annually (Kg)
0.1	41.6	5.2
1	416	52
5	2080	260
10	4160	520

Product Demands
- Shift to smaller production reactors
- Process redesign to fit existing facility infrastructure

Assumptions:
2 Bioreactor production trains
1 Purification train
7 day thaw rate
No operational, Facility, Equipment bottlenecks

Impact of Harvest Titer on Facility Throughput



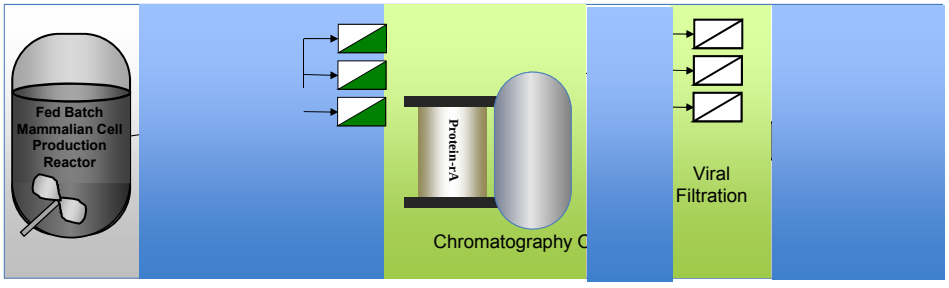
Single Product in three Large-scale Biomanufacturing Trains and Two Purification Suites

Presentation Overview

- Reshape Conventional Biologic Manufacturing processing steps to address
  1. Bottlenecks associated with increased Production time and Downstream operations constraints
  2. Process Space Compression to address increased titers
  3. Increase Throughput Capacity
  4. Cost Pressures

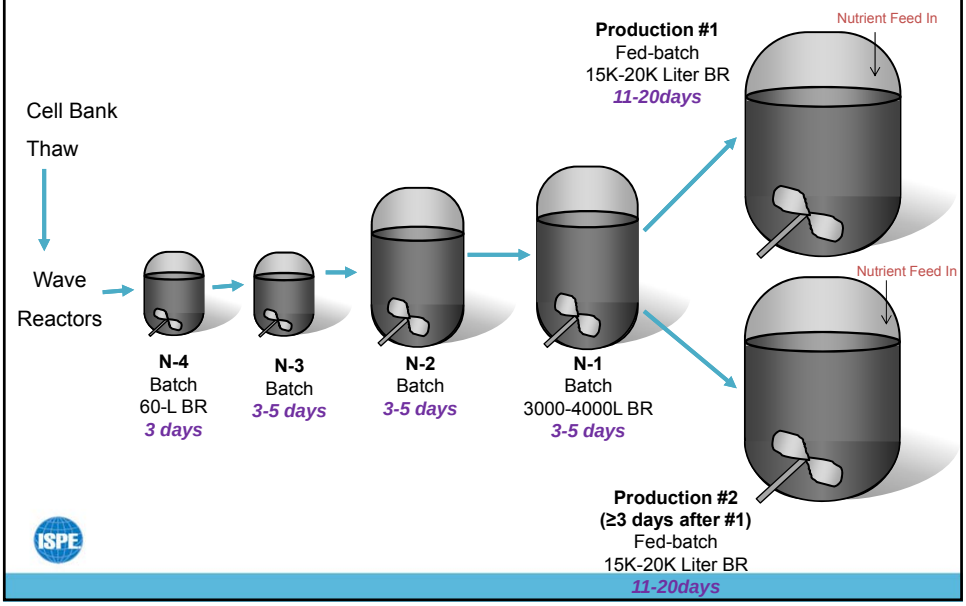
- Review Technologies / Capabilities that address the above drivers
  - Production Reactor throughput
  - Downstream Capacity and Process Compression

Fed-Batch Mammalian Process

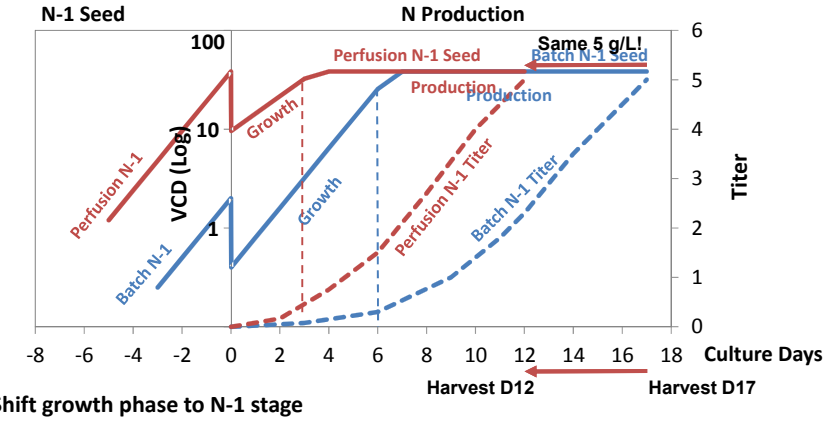


- Typical mammalian cell culture process including innoculum train, fed-batch production reactor
- Cell clarification via centrifugation, microfiltration and/or depth filtration
- Initial Capture Chromatography (Bind-Elute) for majority of Purification
- Secondary Chromatography – Polishing step for product variant, aggregate removal
- Viral Filtration – Robust Virus removal
- Ultrafiltration/Diafiltration - Buffer exchange - Formulation

Typical Large Scale Fed-batch Cell Culture MFG Process



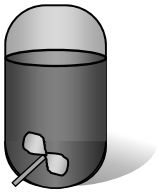
N-1 Cell Retention Debottlenecking



- **Shift growth phase to N-1 stage**
  - Very high-seed production cultures to **shorten culture duration**
  - **More batches** in the same amount of time
  - Increase production capacity by more efficiently utilizing the ratio between N-1/N stage bioreactors
  - ***No changes in production (N) media or volumetric capacity***

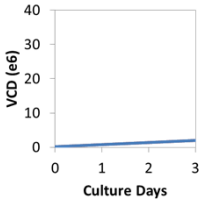
N-1 Bioreactor Modes of Operation

Batch N-1

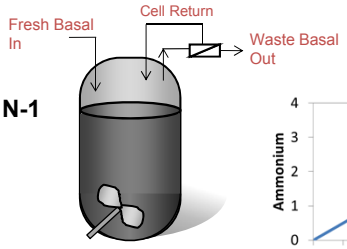


Waste Accumulates

No New Nutrients Added

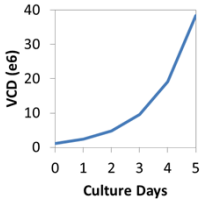


Perfusion N-1

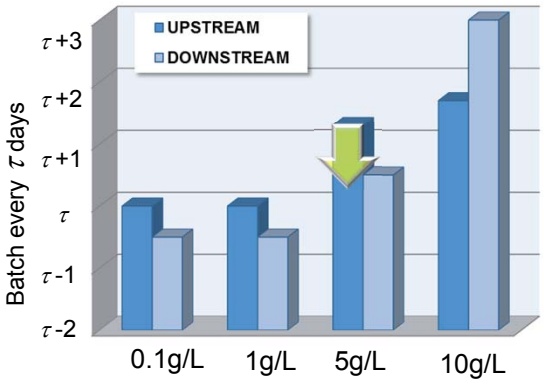


Waste Removed

Cells Retained
Nutrients Added



Big Impact on Manufacturing Capacity



- Assumptions
  - Campaign length is 1year, maximum number of batches per 365-day campaign slot, 2-day turnaround time per bioreactor

Limitations of Current Purification Platform

Capacity: process volumes limit throughput for titers > 4-5 g/L

- Resin binding capacity
  - Large columns x multiple cycles = large volumes
- Protein A most concerning
  - 30- 40 g/L capacity
  - Polishing steps flow-through mode

~5 g/L at 20,000L Scale

1.6 m Protein A column (400L resin, 6 cycles, 45,000 L buffer)

- Protein A eluate volume ~ 7100 L
- Polishing Chrom eluate volume ~10,500L

Buffer Volume Constraints

Process Intermediate Volume Constraints

High Titer Processing: Strategies Capture

New Capture Resins that provide improved capacity and/or Productivity

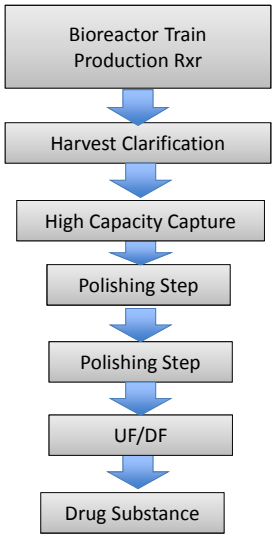
Alternative non ProA Capture resins eg IEX, HyperCel

Factors that drive one technology over another:
(1) COGs
(2) Platformability,
(3) Scalability,
(4) Facility/Engr Retrofit,
(5) Validation-complexity,

Alternative technologies (eg Precipitation, Expanded Bed)

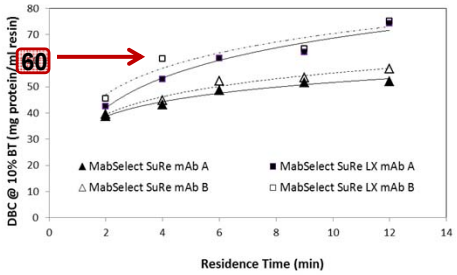
High Capacity Process Alternative Capture Step

- Protein A improvements
  - New suppliers offering lower cost
  - New higher binding capacity resins
  - New modes of operation
- Protein A Replacement
  - B/E mode followed by one or two polishing F/T steps
  - ≥70 g/L dynamic binding capacity to reduce process volumes by ≥ 2X
  - Better resin cleaning and lifetime
  - Eliminate high cost of protein A



High Capacity Resins: Protein A

- For a high titer, shorter duration production bioreactor, the Protein A capture step with ~ 35-40 g/L loading capacity has been identified as a potential throughput bottleneck
  - Many column cycles
  - Large buffer requirements
  - Large intermediate process volumes
- Process modeling has shown that increasing capture column binding capacity to 60 g/L combined with buffer concentrates will alleviate potential bottleneck



Ghose et al., Biotech Progress, 20(3), 2004

Leverage higher capacity resins:

MAbSelect SuRe LX
vs
MabSelect SuRe

Maximizing Capacity on Protein A

MAbSuRe LX

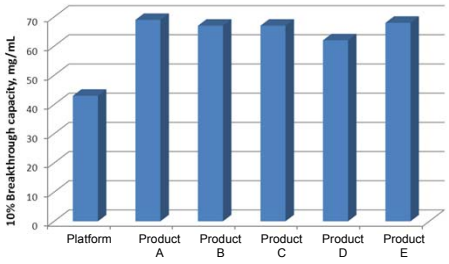
New higher capacity version of resin

Dual flow rate operation

Stepping down flowrate during load optimizes for mass transport

DBC (10 % BT) of 60-70 g/L

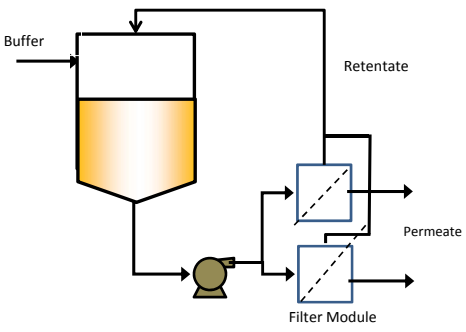
Comparable performance with a ~ 50% increase in binding capacity



Product	Resin	Load g/L	Yield %	HCP ppm	Pr A ppm
A	CONTROL	35	> 95	500	2.1
	SuRe LX	55	> 95	488	3.2
B	CONTROL	35	> 95	300	2.5
	SuRe LX	55	> 95	690	4.6

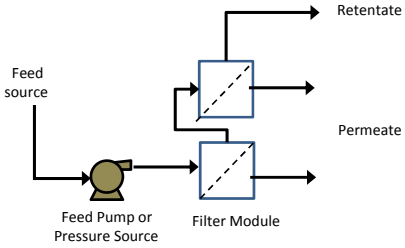
Tangential Flow Filtration Solution

Batch TFF



- Membrane modules in-parallel
- Concentration over time; requires several membrane passes
- Product held in recirculation vessel

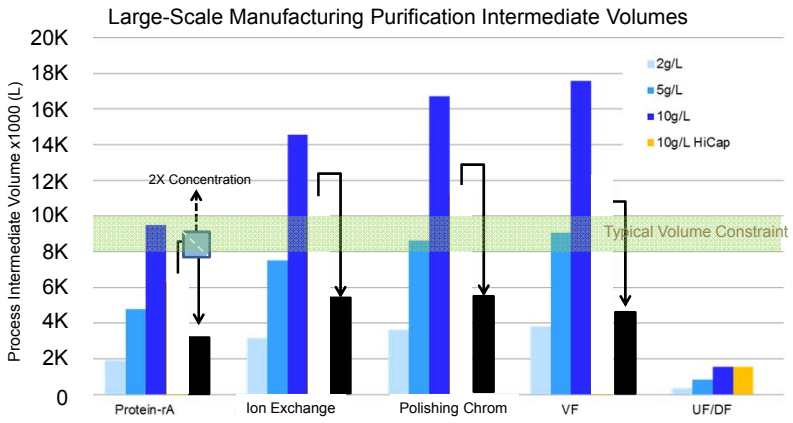
Single-Pass TFF



- Membrane modules in-series
- Concentration over membrane length in a single pass
- No recirculation vessel or feed pump required

- Lower cost, smaller footprint
- Uses conventional UF modules and could be fully disposable
- Higher recovery, lower shear
- Allows in-line operation

Downstream Process Intermediate Volumetric Constraints



- Chromatography Improvements and SPTFF enable HT Processing
  - Reduces downstream volumes in a platform process (FT columns)
  - Facilitate high titer MFG processes (> 5g/L) within existing MFG constraints

Effect of Using Buffer Concentrates

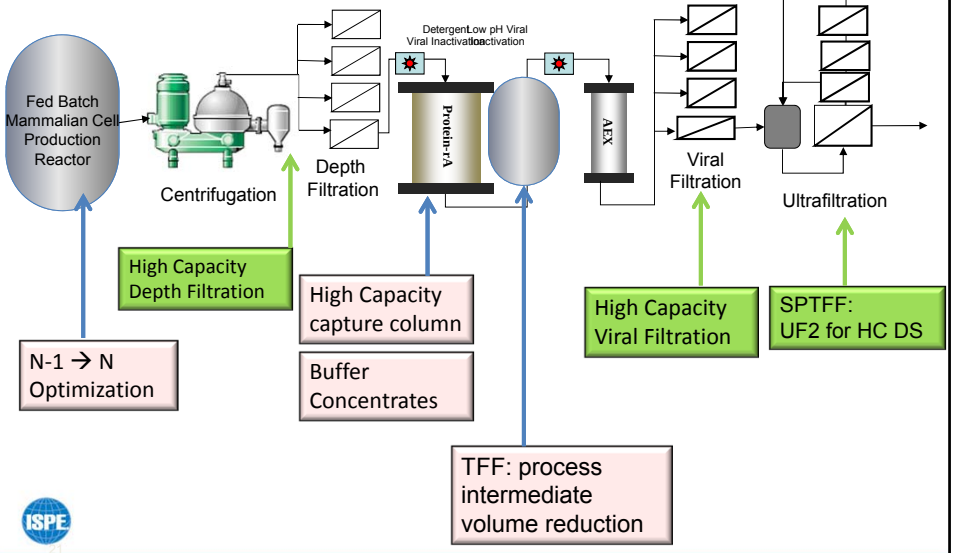
10g/L Harvest Conditions

- Case 1 – All chromatography buffers at 1x
- Case 2 – All chromatography buffers at 5x

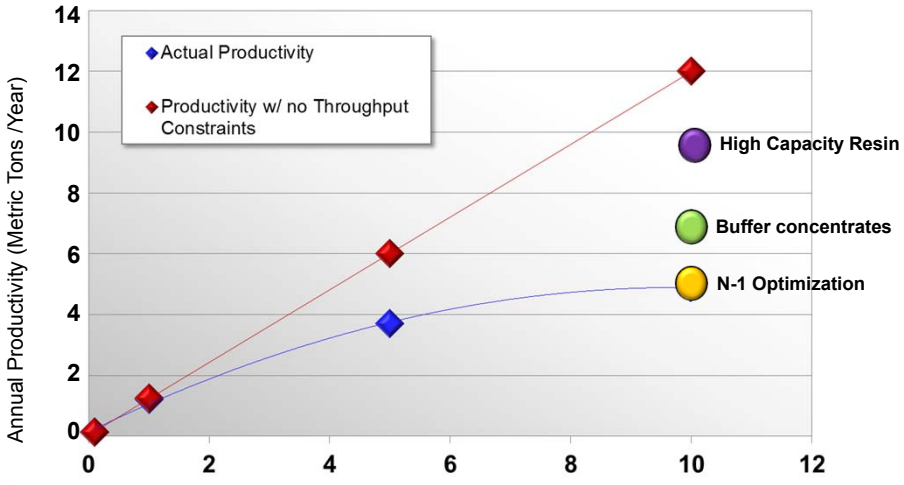
Case	Total # of Buffer Preps	Purification Cycle Time (days)	Upstream Cycle Time (1 train) (days)
1	50-60	τ	τ - 1.5 days

Without concentrates, Purification becomes the bottleneck

Integrated High Capacity Upstream Downstream processing

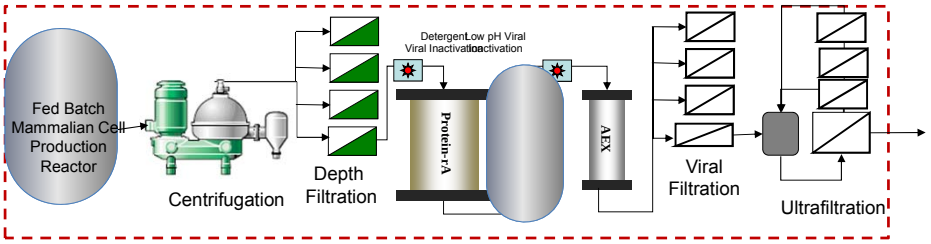


Technology changes on Facility Throughput



Single Product in three 15K Trains and Two Purification Suites

Economic Analysis

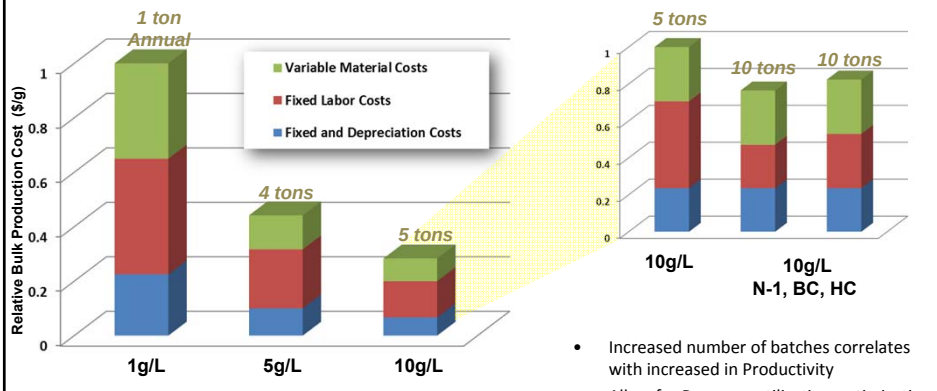


Scope:

Includes processes between the LSM Bioreactor and the Final Bottling Step

23

Production Cost Contributions



- Increased number of batches correlates with increased in Productivity
- Allow for Resource utilization optimization reduce COGs by 23%

Assumptions:

- Model current Purification technology
- Same facility Fit
- Enhanced bioreactor turnaround
- All analysis assumed no labor or utility constraints

Summary

- Facility bottleneck for 1-5g/L Fed batch processes at large scale is production bioreactor (with three trains). Shift in Bottlenecks occur at Downstream as one approached 10g/L
- Integration of advances in N-1 Perfusion, High capacity resins, Buffer concentrates, and intermediate volume reduction allows for throughput increase by 2x as compared to no change in technology
  - Allow the avoidance of expanding facility Footprint
- As annual output and scale increase with titer increase, the relative importance of different cost categories are expected to change
  - Overall cost of goods/ gram product decreases by >70%

Acknowledgements

- N-1 Perfusion:
  - William Yang
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- High Capacity Resins:
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- SPTFF:
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  - Chris Antoniou
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