ThermoFisher SCIENTIFIC

Devising Methods to achieve an efficient cell culture process in Biomanufacturing

Surendra Balekai
Sales Manager, BPP APAC
Thermo Fisher Scientific

November 26, 2012

The world leader in serving science

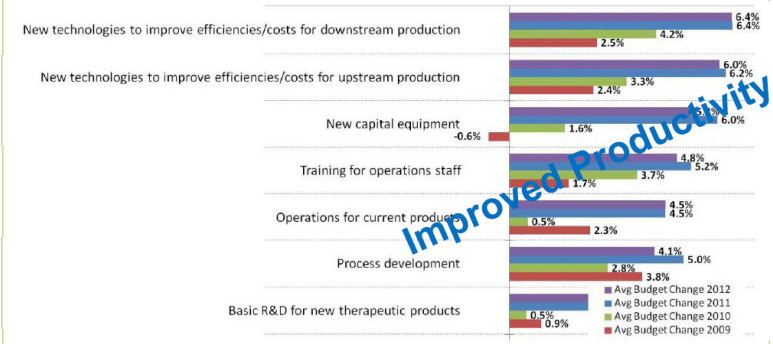
BioPlan associates, inc.

Biopharma Industry Today

- Biopharma is Hot: >$145 billion
- Growing at 15-18%
- New products, new markets, new growth: Driving investment
- Pipeline Shrinkage? Products in development not significantly expanding
- *This may increase likelihood of product success*

BioPlan associates, inc.

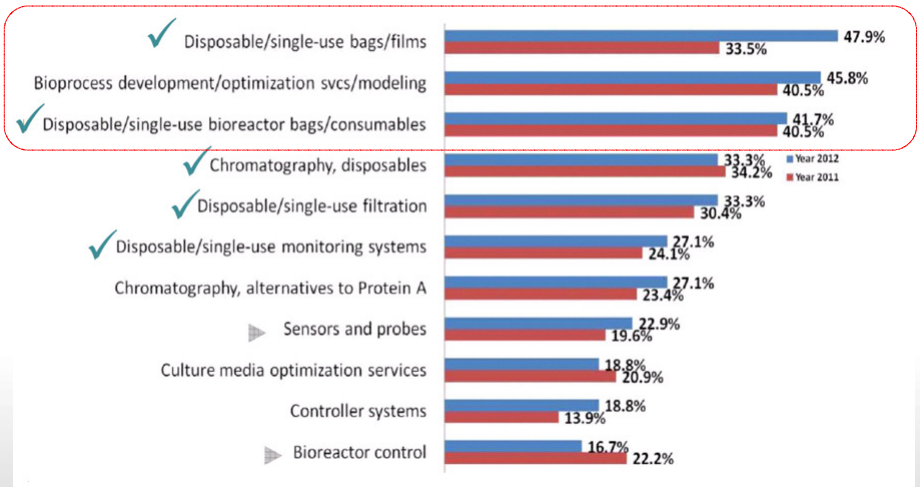
Change in Biomanufacturers' Budgets 2009-12



Improved Productivity

ThermoFisher SCIENTIFIC

New Technologies (Top 11 areas)



ThermoFisher SCIENTIFIC

Unique Approach to Media Optimization

Metabolic Pathway Design™ (MBD)

Method for optimizing media performance by balancing waste and nutrients

Factorial Approach to Design

Investigate relationships based on input factors and output responses

Industry Leading Approach

Combining rigorous theoretical and empirical design principles to developing and optimizing media that exceeds customer expectations

ThermoFisher SCIENTIFIC

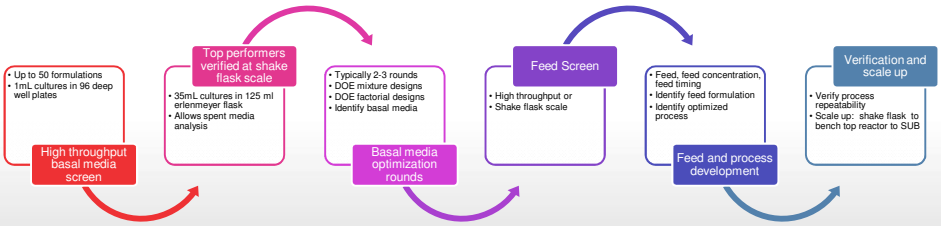
Metabolic Pathway Design™ Overview

A rigorous process for optimizing media performance to maximize cell yield

The Benefit

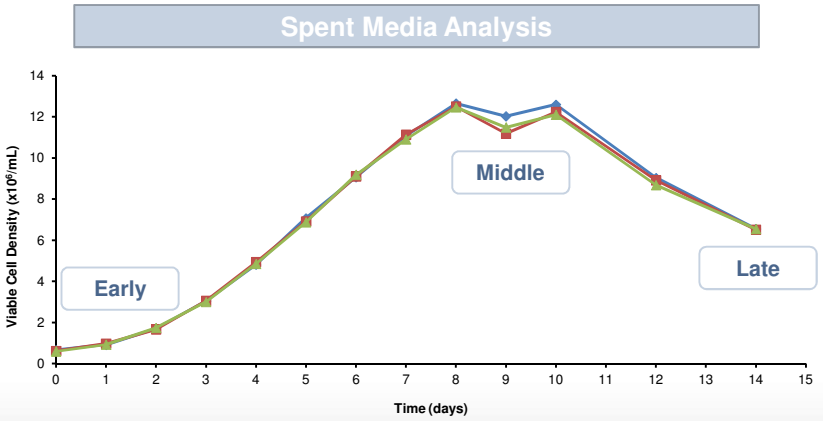
- Drive performance through customized formulations
- Clonal variations of cell lines provides the perfect opportunity to maximize productivity
- Find the right balance of nutrient supply vs. metabolic waste and improve cell yields

The Process



ThermoFisher SCIENTIFIC

Metabolic Pathway Design™ In Use



- Observe media component flux throughout **complete growth cycle**
- Balance waste buildup with nutritional demand
- Adjust formulation to accommodate component deficiencies or buildup

ThermoFisher SCIENTIFIC

Factorial Approach to Design

Overview

- Investigate relationships
  - Input factors
  - Output responses
- **Full factorial designs are unmanageable**
  - #Levels$^{\#Factors}$ = Factorial points
  - CD media can have up to 100 individual components
  - Analysis at 2 levels without replication
    (2^{100}= 1,267,650,600,000,000,000,000,000,000,000)
- **Infeasible due to lack of technology and insufficient resources**

Full Factorial Design Experimentation not Feasible

ThermoFisher SCIENTIFIC

Empirical and Factorial Approach to Optimization

Manageable factorial approaches

- Grouping 'classes' of components
- Limiting factors studied to 'key' components
- Metabolic pathways and effects of factors present

DOE factorial approach

- Analyze levels of components that are already known to be essential
- Identify components that are not relevant

Metabolic Pathway Design

- Balance nutrient supply to achieve high cell viability and productivity
- High throughput screening for much larger design space
- Higher degree of replication for each treatment
- Drive performance through customized formulation for specific cell line applications
- Formulating for nutritional demand

ThermoFisher SCIENTIFIC

Media Optimization Capabilities

- **Culture capabilities**
  - Multiwell to 2000 L bioreactor
  - Standard and high throughput
  - Comprehensive disposable technologies
- **Library of relevant formulations**
  - Reference media
  - Buffers
  - Process supplements
- **Highly qualified raw materials**
  - Full specifications
  - Traceability
  - Change control
- **GMP-like pilot production service**
  - Rapid Response Production™ Service

Comprehensive reference cell lines

Bacteria
- E. coli
- Clostridium sp

Yeast
- Saccharomyces sp.
- Pichia sp.

ThermoFisher SCIENTIFIC

Optimizing Media Case Study

CASE STUDY: TOP 10 GLOBAL PHARMACEUTICAL COMPANY

- High cell density and productivity for CHO clone
- Target 2g/L accumulated product
- Animal-free, chemically-defined media formulation that outperforms current media

SOLUTION:

Enhance productivity by 3x

- 3 months to develop final media formulation
- Transferred process to single-use manufacturing

ThermoFisher SCIENTIFIC

Optimizing Feeds Case Study

CASE STUDY: TOP 10 GLOBAL PHARMACEUTICAL COMPANY

- Feed Design of Experiment (DOE) using feed variants to identify best feeds
- Chemically defined medium

SOLUTION:

Enhance productivity by 4x

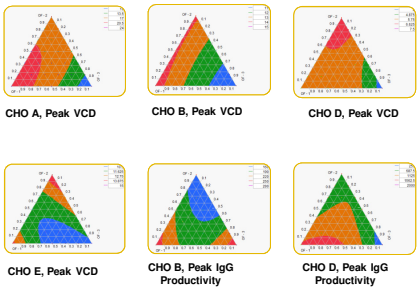
- Customized chemically defined media
- mAb production from 0.4 to 2 g/L
- Doubled peak viable cells
- Twelve-day fed-batch process

ThermoFisher SCIENTIFIC

HyCell CHO Media Development

Leverage Metabolic Pathway Design

- Four different CHO cell lines
  - Large-scale screening 96-Deep-well Plate Studies
  - Shake flask studies
- Identification of three optimal formulations
- Further optimization with large-scale effort applying a DoE mixture design of three optimal formulations
- Final formulation was evaluated at shake level with four cell lines
- Verification in large scale bioreactor runs



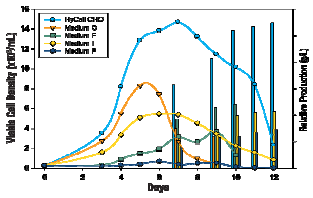
Ternary plots reveal hot spots for promising mixtures using optimal formulations 1,2, and 3. Red and orange areas are most favorable. Mixtures were tested on several cell lines in an attempt to find the most universal blend.

Versatile Performance Across Variety of CHO Clones

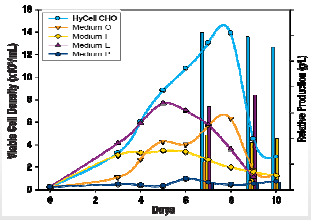
ThermoFisher SCIENTIFIC

HyCell CHO Medium Performance Comparison

CHO-S Cell Line

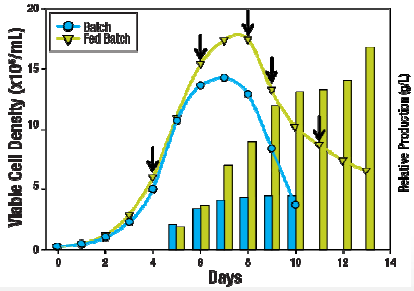


CHO-DG44 Cell Line



Feeds: mixture of Cell Boost 2 and Cell Boost 5 at 6:4 ratio. Feeds of 6% (w/v) concentration were delivered at 10% (v/v) of cell culture at times indicated by arrows

CHO-K1 Cell Line



Peak productivity and VCD show similar trends over deep well plates and shake flasks

ThermoFisher SCIENTIFIC

Expected Bioreactor Capacities

- **What is target molecule and dose required per treatment?**
- **What is the quantity required per Clinical Phase I, II and III?**
- **What is the quantity at commercial scale expected?**
- **What is your expression rate?**
- **What is the overall yield expected?**
- **What is the capacity of the bioreactor required?**
- **Whether to have Single bioreactor of large size or multiples of medium size?**
- **What is the investment required for a Stainless steel vs Disposable?**

ThermoFisher SCIENTIFIC

Material Demand Phase I , II, III & Market Size

Required runs at given scale and productivities

	Demand (g)	Bioreactor Volumes (L)	Harvest titer (g/L)		
			1	2	3
			Number of Runs		
Preclinical	50	100	1	0.5	0.3
Phase I	250	100	5	2.5	1.7
Phase I	250	250	2	1	0.7
Phase II	1000	250	8	4	2.7
Phase II	1000	1000	2	1	0.7
Phase III	10000	2000	10	5	3.3
Market Size	250000	10000	50	25	16.7
Market Size	250000	5000	100	50	33.3

DSP Yield - 50%

ThermoFisher SCIENTIFIC

Why Choose SUT?

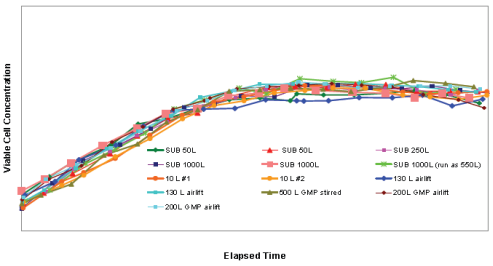
Eliminate Contamination Risks
- Industry estimates of product loss due to contamination ~ 5-20%

Lower Initial Investment Costs
- 74% Capital Savings

Lower Operating Costs
- 90% Water Reduction (Process and WFI)
- 45% Faster Changeover
- 40% Energy Reduction

Business Continuity
- Faster speed to market (concept to production)
- Campaign flexibility

SUT performance compared to stainless steel

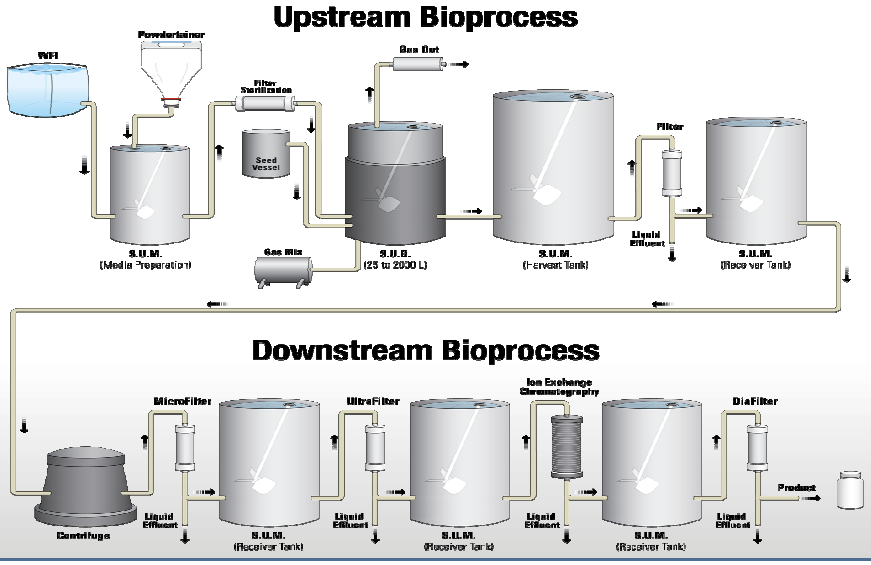


Cell growth in fed-batch production mode GS CHO cell type

Single-use bioreactor utilizes stainless steel bioreactor principles to provide seamless transition to the single-use model

ThermoFisher SCIENTIFIC

Applications of SUT in Bioproduction



ThermoFisher SCIENTIFIC

Single-Use Bioreactor BPC

Port labeling on bags and cables

Single-use connectors

Probe belt for classical & single-use sensors

Pressure sensor

Open pipe sparger

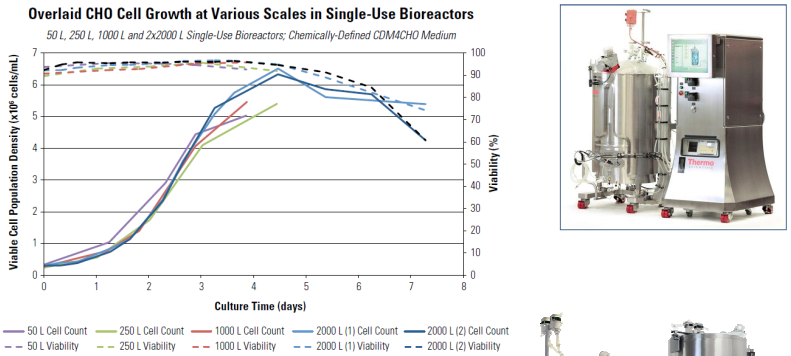
Frit sparger

Optional sample manifold

- CHO Cells
- Hybridomas
- Myelomas
- HEK 293
- PER.C6®
- BHK 21
- Vero
- MDCK

ThermoFisher SCIENTIFIC

Single-Use Bioreactor (S.U.B.)



ThermoFisher SCIENTIFIC

Different Cell lines and Scalability

Evaluation of scale up **performance from 50 L to 1000 L** to show comparability of cell culture and quality of product to stainless steel systems

Lonza

Summary of overall experience to date with SUB's

- We have executed around 40 runs in the pilot SUB's
- Runs include a mixture of cell line types
  - GS-CHO
    - Lonza CHO process (versions 6 and 7)
    - Customer process drop ins
  - CHO dhfr
  - GS-NS0
  - Human
- Commercial projects in SUB's since mid 2007
- Demonstrated successful scale up and transfer to cGMP 1000 L SUB
- Commercial project currently underway that will pilot in SUB and scale directly to stainless for cGMP manufacture

slide 30

Systems successfully demonstrated:

- ✓ Reproducibility of process
- ✓ Robustness of equipment & process
- ✓ Scalability

ThermoFisher SCIENTIFIC

Fed Batch Process – SP2/0 Hybridoma Cells

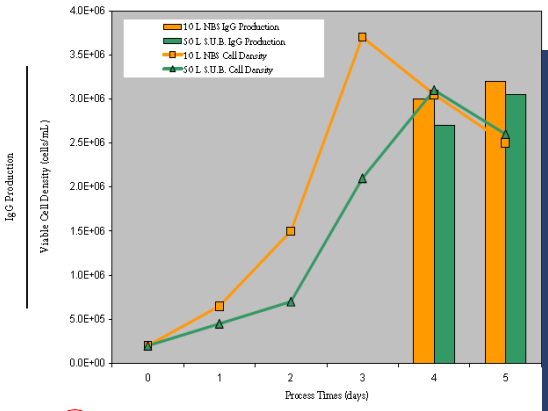


Figure 2: SP2/0 IgG production and cell density data for the 10 L conventional bioreactor and 50 L S.U.B.

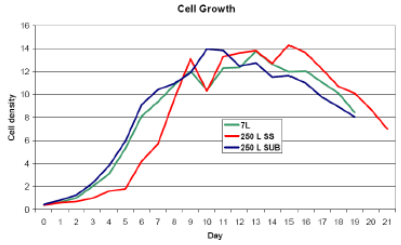
Process Scale and Operating Parameters
50 L Single-Use Bioreactor (S.U.B.)
Working volume (L): 50.0
Agitation rate (RPM): 168
pH set point: 7.0
DO_2setpoint(%): 0.3 lpm (O_2 pulse when needed)
Seed culture: SP2/0 hybridoma (mouse IgG producing)
Cell seed density : $1x10^5$cells/mL

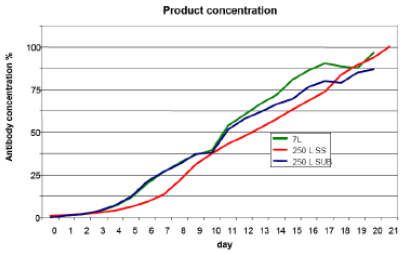
Final cell concentration: $3.11x10^5$cells/mL

ThermoFisher SCIENTIFIC

Fed Batch Process – PER.C6

- Cell line: PER.C6 (Crucell, N.V.) monoclonal antibody producing
  - Process mode: fed batch
  - Scale: 250 L working volume
  - Peak CPD: 1.4×10^7 cells/mL
- Peak product yield: equivalent to stainless steel bioreactor



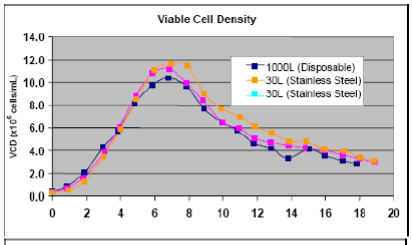


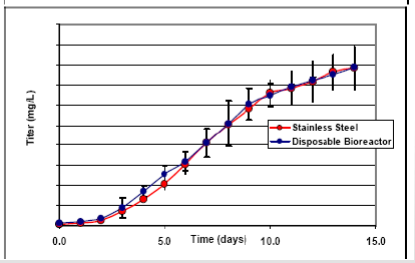
Gerben Zijlstra, DSM, 2007

ThermoFisher SCIENTIFIC

Fed Batch Process- CHO

- Cell line: CHO monoclonal antibody producing
  - Process mode: fed batch
  - Scale: 1000 L working volume
  - Peak CPD: 1.1×10^7 cells/mL
- Peak product yield: equivalent to stainless steel bioreactor





Sadettin Ozturk, Centocor, 2007

ThermoFisher SCIENTIFIC

Perfusion Process – CHO Cells

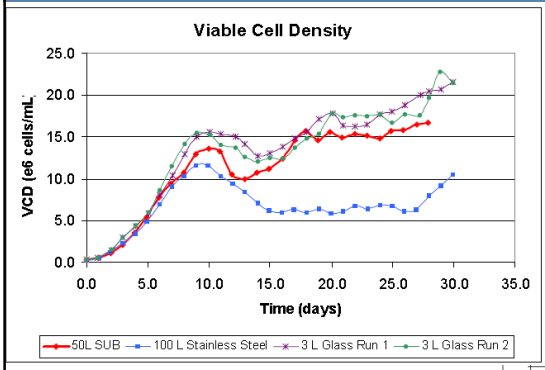
Process Scale and Operating Parameters
50 L Single-Use Bioreactor (S.U.B.)
Working volume (L): 30.0
Run duration : 28 days
pH setpoint: 7.4
DO_2 setpoint(%): 40%

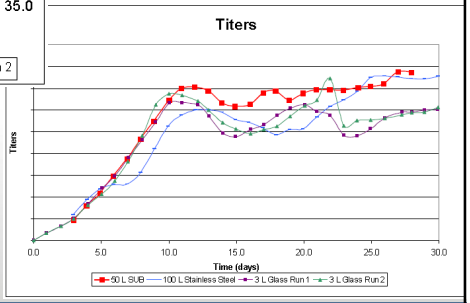
Perfusion Profile:
–Day 2: 0.2 bioreactor volumes/day
–Day 3: 0.4 bioreactor volumes/day
–Day 4: 0.8 bioreactor volumes/day
–Day 5 and on: 1.0 bioreactor volumes/day

Nicole E. Richardson & Barbara Chiang
Centocor R&D

ThermoFisher SCIENTIFIC

Perfusion Process – CHO Cell





Nicole E. Richardson & Barbara Chiang
Centocor R&D

ThermoFisher SCIENTIFIC

Quality Aspects

Lonza

Product Quality Evaluation

Purity	Identity	Impurity profile
SDS PAGE (reduced and non-reduced)	icIEF	HCP Western blot
GP HPLC	Glycosylation profile	Protein A ELISA
	ESI-MS	DNA by qPCR

ThermoFisher SCIENTIFIC

Single Use Technology

Speed	Cost	Quality
No CIP or SIP	Reduced cleaning costs	Decreased risk of cross-contamination
Less complex validation	Reduced utility costs	Less effort, less mistakes
Less downtime	Less space required	Reduced need for audit
Quicker set-up	Less engineering complexity	Reduced QC resources per cycle

Higher quality, with increased throughput, and reduced cycles time at a lower cost.

ThermoFisher SCIENTIFIC

Main Industry Drivers for SUT

- Patent cliff creating a market for biosimilars and biobetters
- Pandemic flu vaccine driving adoption of faster, more easily reproducible culture methods (away from egg-based)
- Emerging markets pressing demand for medicines and evolving regulatory framework for in-country manufacturing
- Pharmacogenomics (personalized medicine) will require smaller batches for production
- Contract manufacturing organizations becoming a viable and reliable outsourcing option

Bottom line:

Our customers are increasingly demanding single-use solutions in an effort to reduce cost and increase flexibility in a more competitive market with more market entrants (countries & manufacturers) and fewer patent protections

Future of SUT

- SUT have quickly become a cost-effective replacement for stainless steel model for many reasons
- Continuing demands for SUT driven by biopharma customers and new market entrants to biological production
- Smaller batch sizes due to flexibility of SUT
- Single use manufacturing of the future models are becoming the norm
- Better alignment between biopharma manufacturers and SUT suppliers to eliminate lead-times and customize solutions specific to customer needs

Your Partner In Science





ThermoFisher SCIENTIFIC

The world leader in serving science

Committed to our customers' goals for better results and greater productivity