Thermo Scientific Metabolic Pathway Design Process – Cell culture media and process optimization approaches for optimal biotherapeutic production

May/June 2013
Agenda

- Introduction
- Media Capabilities Overview
- Media Services
- Case Studies
- Conclusion
Cell Culture Media

- State-of-the-art cGMP manufacturing facilities on two continents
- Liquid and powder options available
- Highly customized
- Supports a large range of cell culture platforms
- Segregated manufacturing facilities for animal origin products
- Sample media formulations available within 7 days
- Shipped in packaging validated to meet ISTA-2A and ISTA-1E standards

Supported cell culture platforms

- CHO Cells
- Hybridomas
- Myelomas
- NS0
- HEK 293
- Crucell PER.C6™ cells
- Vero
- MDCK
- BHK21
- Gene Therapy
- Insect Cells
- Viral Vaccines

Your expert partner for media collaboration
Dry Powder Media Manufacturing

**Traditional milling and blending**
- Ceramic Ball Milling
- Blending Up to 4,000kg per batch

**Innovative pin milling and blending**
- Dedicated Thermo Scientific™ ADCF™ facilities
- Up to 6,500kg per batch
- Stringent particle size control through micronization
- Maintenance of integrity of temperature-sensitive components
- Homogeneity of components in formulation

*State-of-the-art milling and blending*
Liquid Media Manufacturing

Manufacturing options
- Traditional stainless steel
  - Consistent and redundant processes in the U.S. and UK
- Innovative single-use technologies
  - Located in the U.S.
  - Eliminates CIP/SIP requirements
  - Rapid turnaround

Filling and packaging options
- Lot sizes ranging from 100 to 10,000L
- Packaged and shipped in ISTA-2A and ISTA-1E secure containers
- Available in 1L bottles and 1 to 500L bioprocess containers
- Custom packaging to suit your needs
Media Services

1. **Media and Feed Formulation**
   - Formulation enhancement
   - cGMP Manufacturability
   - Use of Thermo Scientific™ Metabolic Pathway Design™ principles

2. **Media Optimization and Process Development**
   - Optimize cell density and productivity
   - Small to large scale
   - Spent media analysis
   - Raw materials and therapeutic product analysis

3. **Rapid Response Production**
   - Rapid prototyping of media and bioprocess containers
   - Non-GMP
   - Shipped within seven days of order

*Customized solutions to help you scale to cGMP*
Media Optimization Capabilities

- Culture capabilities
  - Multiwell to 2,000L 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
  - Thermo Scientific™ Rapid Response Production™ Service

Comprehensive reference cell lines

- CHO
- NS0
- PER.C6
- Hybridoma
- HEK 293
- BHK21
- Vero
- MDCK
- Sf9, High Five, D.mel
Our Unique Approach to Media

Metabolic Pathway Design Process
Our proprietary 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 for Media
Combining rigorous theoretical and empirical design principles to developing and optimizing media that exceeds customer expectations
Cell Culture Media Optimization Overview

Goals for an efficient upstream bioprocess

- Cell population growth rate
- Viable cell density
- Specific production rate
- Maximum peak viable cell population density
- Rapid process turn-around time

Cell culture conditions that drive efficiency in bioprocessing

1. Clone
2. Medium
3. Feeds
4. Process

*Cell culture medium and feeds are significant factors*
Media and Feed Formulation

Chemically defined media and feeds are complex nutrient formulations

- Amino acids
- Vitamins
- Trace elements and salts
- Carbohydrates
- Lipids
- Growth factors and intermediates
- Antioxidants
- Buffers

Complex interaction between

- Media and feed nutrients
- Metabolic pathways
- Stage of bioprocess
Thermo Scientific Metabolic Pathway Design™ Process

*Balance nutrient supply* to achieve high 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
  - Improve cell yields for *greater scalability*
  - Focus development on *productivity* and *quality* (e.g., MAbs, etc.)
- Formulating for *nutritional demand*
  - Evaluate component-specific effective *dose*
  - Provide complex lipids for *enhanced stability* and *delivery*

*Optimization through metabolic pathway design*
Overview

A rigorous Metabolic Pathway Design process for optimizing media performance to maximize cell yield

Our expertise
• Comprehensive reference cell lines and reference media formulations
• Over 40 years of experience in cell culture

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
• Up to 50 formulations
• 1mL cultures in 96-deep-well plates

Top performers verified at shake flask scale
• 35mL cultures in 125 mL erlenmeyer flask
• Allows spent media analysis

Typically 2-3 rounds
• DoE mixture designs
• DoE factorial designs
• Identify basal media

High throughput or
• Shake flask scale

Feed screen
• Feed, feed concentration, feed timing
• Identify feed formulation
• Identify optimized process

Feed and process development
• Verify process repeatability
• Scale up: shake flask to bench top reactor to S.U.B.
Media Optimization Capabilities

### Spent Media Analysis and Biochemistry
- Cell density, viability, and size distribution
- Metabolites and waste products: glutamine, glutamate, glucose, lactate and ammonia
- Quantification of amino acids, vitamins, and IgG
- Elemental analysis of metals and ions, media constituents, and unknown IDs

### High Throughput Analysis
- DoE approach
- Media, feed formulation, and optimization
- Automated cell density, viability, and productivity measurements

### Flow Cytometry and Cell Characterization
- Various cellular analyses, including transfection efficiency, cell cycle analysis, etc.

### Cellular and Process Analytics
- pH, Osmo, FTNIR, turbidity, and filterability
- ELISA, PAGE, NIR, and protein assays

#### Spent Media Analysis

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Typical Frequency</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolites and waste products (glutamine, glutamate, glucose, Lactate and ammonia)</td>
<td>Daily</td>
<td>Nova Bioprofile</td>
</tr>
<tr>
<td>Amino Acids</td>
<td>T0, mid culture, late culture</td>
<td>HPLC</td>
</tr>
<tr>
<td>Vitamins</td>
<td>T0, mid culture, late culture</td>
<td>HPLC</td>
</tr>
<tr>
<td>Trace elements</td>
<td>T0, mid culture, late culture</td>
<td>ICP</td>
</tr>
<tr>
<td>Lipids</td>
<td>Varies</td>
<td>GC</td>
</tr>
</tbody>
</table>
Optimization Tools

- **Culture Monitoring**
  - Cell density, via. and size
  - Product quantity and quality
  - Physicochemical (e.g., pH)
  - Amino acid levels
  - Metals levels (e.g., copper)
  - Vitamin levels (e.g., folate)
Optimization Tools – 50L S.U.B.

Cell Growth in the Dual Sparge 50L S.U.B. Using a Chemically-Defined, Serum-Free Medium

Optical Sensor Evaluation

VCPD (cells/mL) TCPD (cells/mL) Viability Glutamine (mM) Glucose (g/L) Lactate (g/L) Ammonium (mM)
• Observe media component flux throughout complete growth cycle
• Balance waste buildup with nutritional demand
• Adjust formulation to accommodate component deficiencies or buildup
Optimizing Media Case Study

CASE STUDY

• High cell density and productivity for CHO clone
• Animal-derived component-free, chemically defined media formulation that outperforms current media

TARGET

• Goals
  • Enhance viable peak cell densities
  • Develop base media resulting in productivities >1g/L
• Media Design Criteria
  • Media available as both powder and liquid
  • Chemically defined media and feeds preferred
  • Media and feed must be free of animal-derived components
Optimizing Media Case Study

CASE STUDY:

APPROACH

• Screen component groupings and CD basal media formulation library
• HTS and Shaker Flasks
  • Productivity, cell population growth, and viability
  • Metabolic profile
• Best performing conditions were tested in multiple passages
• Final prototypes for bioreactor runs

RESULTS

• ADCF and CD media
• Less than 3 months to develop final media formulation
• Transferred process to single-use manufacturing
• >2g/L accumulated product

Enhanced productivity by 3x
Optimizing Feeds Case Study

CASE STUDY

• Standard batch cultures
• Peak cell yield: $4 \times 10^6$ cells/mL
• MAb production: ~0.4g/L
• Process time: 8 days

TARGET:

• Goals
  • Enhance viable peak cell densities
  • Develop media and fed batch process resulting in productivities greater than 1 gram per liter
• Media Design Criteria
  • Media available as both powder and liquid
  • Chemically defined media and feeds preferred
  • Media and feed must be free of animal-derived components
Optimizing Feeds Case Study

CASE STUDY

APPROACH
• Performance in basal media
  • VCD, product yield, spent media analysis (metabolites and waste products, amino acids)
• Feed design of experiment (DoE) using feed variants to identify best feeds
  • High-throughput feed screen
  • Analysis of top contenders in shake flasks

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

Enhanced productivity by 4x

Growth and productivity of a CHO derived cell line in optimized basal medium, comparing batch to fed-batch culture modes. Optimized feed was delivered at times indicated. Cell Boost 2 on days 3, 5, 7 and Cell Boost 5 on days 4, 6, 8
Conclusion

• Metabolic Pathway Design process has been invaluable in understanding the metabolic requirements of cells and has been successfully applied to develop clone-specific high performing bioprocess cell culture media and feeds.
Why Choose Thermo Scientific Media?

High Quality Standards
- ISO 9001 (2008)
- Eudralex Annex 1
- Medical Device cGMP 21 CFR820

Robust Service Offering
- Media and feed formulation
- Media optimization and process development
- Rapid response production

Optimized for your Process
- Animal-derived component-free
- Serum-free media
- Protein-free media
- Chemically-defined media
Your Partner In Science

Committed to your goals for better results and greater productivity