Optimized Feed Strategies for Increased Titer and Product Quality

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Agenda

Pain points in fed-batch culture

Addressing pain points

• Nutritional and dilutional gaps
• Process considerations
• Importance of product quality
• Factors that can influence product quality
• Latest advance in high-throughput glycan testing

Conclusions
Pain Points in Fed-Batch Culture

Nutritional gap
- Engineered cells have high nutritional demands
- Providing the right nutrients at the right time
- Matching basal medium and feed

Dilutional gap
- Dilution impact of feed addition
- How concentrated can you make the feed to be able to feed more?

Process
- High/low pH feeds add to preparation complexity and time
- Pick clones in a system that reflects ultimate process

Desired end point
- Cell density, product titer, product quality
- High cell density may not correlate with high titer or with desired critical quality attributes
Engineered Cells Have High Nutritional Demands

![Graph showing the nutritional demands of engineered cells. The x-axis represents run time in days, and the y-axis represents viable cell density (10^6 cells/mL) and EPO titer (mg/L). Concentrations of Arginine, Asparagine, Cysteine, and Glutamine are also shown, decreasing over time.](image-url)
Balanced Formulations Drive Performance

Sample target process

Perform spent media analysis
Analyze growth/titer

Calculate consumption rates

Analyze nutritional pathways

Optimized balanced formulations

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Consumption Rate (mmol/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-ALANINE</td>
<td>-0.251</td>
</tr>
<tr>
<td>L-ARGININE</td>
<td>0.055</td>
</tr>
<tr>
<td>L-ASPARAGINE</td>
<td>0.054</td>
</tr>
<tr>
<td>L-ASPARTIC ACID</td>
<td>0.045</td>
</tr>
<tr>
<td>L-CYSTEINE</td>
<td>0.083</td>
</tr>
<tr>
<td>L-GLUTAMIC ACID</td>
<td>0.055</td>
</tr>
<tr>
<td>GLYCINE</td>
<td>0.068</td>
</tr>
<tr>
<td>L-HISTIDINE</td>
<td>0.024</td>
</tr>
<tr>
<td>L-ISOLEUCINE</td>
<td>0.048</td>
</tr>
<tr>
<td>L-LEUCINE</td>
<td>0.066</td>
</tr>
<tr>
<td>L-LYSINE</td>
<td>0.063</td>
</tr>
<tr>
<td>L-METHIONINE</td>
<td>0.036</td>
</tr>
<tr>
<td>L-PHENYLALANINE</td>
<td>0.061</td>
</tr>
<tr>
<td>L-PROLINE</td>
<td>0.057</td>
</tr>
<tr>
<td>L-SERINE</td>
<td>0.07</td>
</tr>
<tr>
<td>L-THREONINE</td>
<td>0.05</td>
</tr>
<tr>
<td>L-TRYPTOPHAN</td>
<td>0.039</td>
</tr>
<tr>
<td>L-TYROSINE</td>
<td>0.062</td>
</tr>
<tr>
<td>L-VALINE</td>
<td>0.064</td>
</tr>
</tbody>
</table>
Benefit of Rationally Integrated Optimization

**Cell Line/control process**
- CHO-K1SV–high IgG expression
- Modified catalog base medium
- Proprietary feed/process

**Desired outcome**
- Improved titer
- Proprietary base medium
- Formulation access
- Stay with current feed/process

Modification of basal medium alone was **insufficient to improve titer**

Process Intensification Addresses Nutritional and Dilutional Gaps

Higher total productivity by maintaining $q_p$
Sustaining $q_p \rightarrow$ Increased $Q_p$ in Matched Basal/Feed Media

Elimination of nutritional gap = ~2-fold titer increase
Granulated Format Improves Process and Productivity

**Processing time cut in half vs. DPM**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral pH</td>
<td>pH range: 6.5–7.1</td>
</tr>
<tr>
<td>Super-concentrated feeds</td>
<td>Target: 150–200 g/L</td>
</tr>
<tr>
<td>Simple to prepare</td>
<td>Just add water</td>
</tr>
<tr>
<td>Faster hydrating</td>
<td>≤90 min</td>
</tr>
<tr>
<td>Flexible in its use</td>
<td>Add less water to concentrate</td>
</tr>
</tbody>
</table>

**Fluid granulation**

- **Spraying**
  - Trace liquids
  - Powder
- **Moistening**
  - Liquid bridge
- **Solidifying**
  - Solid bridge
- **Finished granule**
  - AGT granule
Increased Titer with Highly Concentrated Feeds

30–40% titer increase by eliminating the dilutional gap
Clone Ranking Varies with Process

Top 10 MabB Clones in SFB & FB

<table>
<thead>
<tr>
<th>Clone ID</th>
<th>Titer (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>34F05</td>
<td>Simple Fed-batch</td>
</tr>
<tr>
<td>54A05</td>
<td>Fed-batch 1</td>
</tr>
<tr>
<td>02B07</td>
<td>Fed-batch 2</td>
</tr>
<tr>
<td>05B09</td>
<td></td>
</tr>
<tr>
<td>05D09</td>
<td></td>
</tr>
<tr>
<td>25F10</td>
<td></td>
</tr>
<tr>
<td>05C07</td>
<td></td>
</tr>
<tr>
<td>60D06</td>
<td></td>
</tr>
<tr>
<td>55F02</td>
<td></td>
</tr>
<tr>
<td>05A03</td>
<td></td>
</tr>
</tbody>
</table>

- Simple Fed-batch
- Fed-batch 1
- Fed-batch 2
**Critical quality attribute:**

“A physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range or distribution to ensure the desired product quality”*

**Examples of critical quality attributes of biotherapeutics:**

- Glycosylation
- Charge variants
- N- and C- terminal structural features
- Fragmentation
- Aggregation

* ICH, ICH Harmonized Tripartite Guideline Q8: Pharmaceutical Development, Step 4 version (August 2009)
Glycosylation is a Key Critical Quality Attribute

**Glycosylation can affect:**

- Protein folding
- Solubility
- Immunogenicity
- Enzymatic activity
- Transport
- Turnover rate

Activity and availability of the protein are impacted by **glycans**
Productivity Increase While Maintaining Quality Profile

No change in key glycan structures
Addressing Media and Process Impact on Glycosylation*

• pH
• DO, pCO₂
• Temperature
• Low glucose/glutamine
• Mn, NH₃, glycerol, nucleotide sugar content
• Cell viability at harvest
• Shear stress
• Perfusion vs. fed batch

* From review by Hossler P, Khattak SF and Li KJ, Glycobiology, 19(9):936-949, 2009
Preventing Ammonia Accumulation → Galactosylation

Day 14: $\text{NH}_4^+$: 9 mM
Day 14: $\text{NH}_4^+$: 6 mM
Day 14: $\text{NH}_4^+$: 4.5 mM
Controlled Conditions in Bioreactors \uparrow Galactosylation

![Graph showing a comparison between Bioreactor and Shake flask. The x-axis represents the biological conditions, while the y-axis represents the percentage (%). The graph indicates a significant increase in galactosylation in the Bioreactor compared to the Shake flask. The legend includes symbols for different conditions: G0F, G1F, G2F, G0, G1, M5.]
Lower Harvest Viability ↑ G0F, ↓ G1F
CHO Transcriptome Comparison

Glycosylation differences between cell lines linked with transcriptome profiles

Increased transcripts in DG44: ALG2, ALG3, ALG9 and ALG12, ALG8 and ALG10, ALG14, and B4GALT5

Higher terminal galactose in DG44
Media/Feed Composition Influences Glycosylation

![Graph showing glycosylation percentages for different feed compositions](image)
Additives Only Provided Partial Modulation of Glycans

An additive approach alone **did not provide the ability to target a desired glycan profile**
Another Approach Was Tried Using Gibco™ GlycanTune™ C+ Total Feed

A

B

Culture time (days)

VCD (x10⁶ cells/ml)

Culture time (days)

IgG titer (% of control)

- Glucose control
- 20% 2X EFC+ Control
- 15% 3X GTC+ Transition day 15
- 15% 3X GTC+ Transition day 13
- 15% 3X GTC+ Transition day 11
- 15% 3X GTC+ Transition day 9
- 15% 3X GTC+ Transition day 7
- 15% 3X GTC+ Transition day 5
Another Approach Was Tried Using GlycanTune C+ Total Feed

The timing of transition from Gibco™ EfficientFeed™ C+ (EFC+) to GlycanTune C+ (GTC+) makes it possible to target specific glycosylation profiles.
Case Study: Modulating Glycan Profile

**Problem**
Customer was satisfied with yield but required product quality modulation to match the innovator molecule (4/12 glycan attributes within an acceptable range).

**Starting point:**
Customer was satisfied with yield but required product quality modulation to match the innovator molecule (4/12 glycan attributes within an acceptable range).

**Solutions**
- Analyze product quality data
- DoE using in-house IP/literature
- Client analyzes PQ
- Evaluate in ambr 15 HT system

**Results**

<table>
<thead>
<tr>
<th>SA2</th>
<th>SA2F</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA1</td>
<td>SA1F</td>
</tr>
<tr>
<td>M5</td>
<td>G0</td>
</tr>
<tr>
<td>G0F</td>
<td>G1</td>
</tr>
<tr>
<td>G1</td>
<td>G1Fa</td>
</tr>
<tr>
<td>G1Fb</td>
<td>G2</td>
</tr>
<tr>
<td>G2f</td>
<td></td>
</tr>
</tbody>
</table>

PQ improvement

**Outcome:** 9 of 12 attributes within range, remaining 3 within 80% of target.
Current Glycan Analysis Solutions

- Single channel (CE & LC) low-throughput (days/96 samples) separation
- Poor data quality of high-throughput methods
- Labor intensive workflow (pipetting, centrifugation, etc.)
- Use of toxic sodium cyanoborohydride chemistry
- Use of vacuum centrifugation steps
- Generic software taking long analysis time
- Non integrated solution from multiple vendors
- Commercial kits with high cost per sample
- No validation support for result/solution
Key Features Desired for a High-Throughput Glycan Analysis Platform

**Easy sample prep**
- Magnetic bead-based sample prep
- Hands-on time <3 hrs for 96 samples
- Fewer pipetting steps
- No sodium cyanoborohydride use
- No vacuum centrifugation steps

**High throughput**
- Sample prep and data of 96 samples in 7–9 hrs

**Resolution**
- Sialylated glycans
- Structural isomers
- Fucose species
- High-mannose species

**Dye labeling**
- Multiple dyes with superior sensitivity

**High sensitivity**
- Analyze as little as 1 µg of IgG

**Low cost**
- Robust instrument and capillaries with low running cost

**Fully integrated**
- Full sample prep, hardware, and software solution
Novel CE-based HT Glycan Analysis System

**Glycan release**
- Fast <1 hr IgG inputs
  - (1.6–50 µg)

**Glycan purification**
- Reproducible & quantitative
  - (Magnetic beads)

**Dye labeling**
- Multiple dyes
  - Reactivity
  - Sensitivity
  - Resolution
  - No dry down

**Data collection**
- Multi-capillary
  - Run-to-run reproducibility
  - Cap-to-cap concordance

**Data analysis**
- Fit for use software
  - Robust alignment algorithms

Complete sample prep and data of **96 samples in 7–9 hours**
Different Dyes for Better Specificity

Separation of major glycans was possible
Conclusions

• Improved volumetric productivity ($Q_p$) can be achieved by use of rationally designed, matched basal and feed media to sustain specific productivity ($q_p$)

• A granulated feed format enables highly concentrated feeds which can further improve productivity and ease of use

• Product quality can be impacted by choice of media, feed, and process conditions

• High-throughput glycan analysis by capillary electrophoresis simplifies and improves the workflow

Future challenges:

• Methods for custom optimization of other critical quality attributes, including charge variant and aggregation profiles

• Rapid, high-throughput, and sensitive methods for analysis of other critical quality attributes
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Caution: For use as a raw material in further manufacturing applications
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For more information on glycosylation, visit thermofisher.com/GlycanTune

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Appendix
Another Approach Was Tried Using Gibco™ GlycanTune™ A+ Total Feed

The timing of transition from Gibco™ EfficientFeed™ A+ (EFA+) to GlycanTune A+ (GTA+) makes it possible to target specific glycosylation profiles. Modulating G0F from 72% down to 30%, while increasing G1F (1 and 2) and increasing G2F.

A. The growth profiles for the DG44 cell in Gibco™ CD OptiCHO™ medium were similar with both EFA+ and with the use of or when transitioning to GTA+.

B. DG44 titer comparison between feeding conditions. 100% titer is the titer of 15% 3X EFA+ on day 16. Titer results indicate that the use of and transition to GTA+ does not negatively affect protein production.
Another Approach Tried with Gibco™ GlycanTune™ B+ Total Feed

The timing of transition from Gibco™ EfficientFeed™ B+ (EFB+) to GlycanTune B+ (GTB+) makes it possible to target specific glycosylation profiles. Modulating G0F from 80% down to 41%, while increasing G1F (1 and 2) and increasing G2F.

A. The growth profiles for the DG44 cell in CD CHO medium were similar with both EFB+ and when transitioning to GTB+. The large error bar at day 10 was due to clumped cells.

B. DG44 titer comparison between feeding conditions. 100% titer is the titer of 15% 3X EFB+ on day 16. Titer results indicate that the use of and transition to GTB+ does not negatively affect protein production.

Also works with other matched basal/feed media