SAFC®
Pharma & Biopharma Raw Material Solutions

White Paper

# Optimizing Media Feeding Strategies

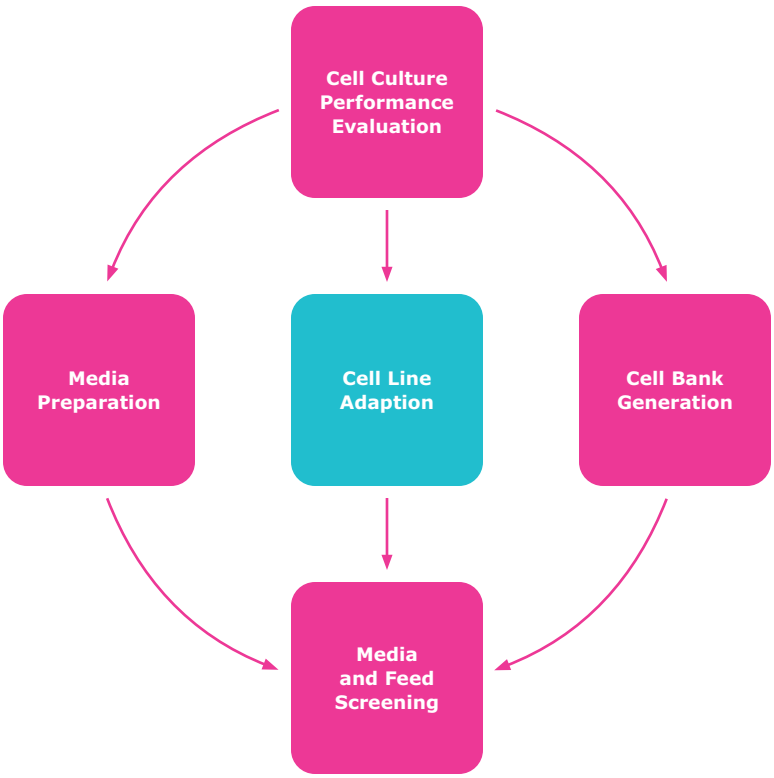
## Guidance for Cellvento® CHO Media Platform (1st GEN)


## Abstract

Performance optimization of upstream mammalian bioprocesses can be accomplished through manipulation of cell line characteristics, adjustment of media and feed formulations, and optimization of process parameters. Cell culture media and feed formulation, and process optimizations are often employed to maximize recombinant product productivity. The purpose of this document is to provide technical guidance and experimental points to consider for bioprocessors conducting upstream bioprocessing development activities for CHO cell-based processes. **Figure 1** below schematically shows the outline of the technical guidance provided in this report.


**Figure 1**.

Schematic of Cell Culture Performance Evaluation Approaches.



## Introduction

Common approaches for biotherapeutics production employing mammalian cell culture utilizes either fed-batch or perfusion modes of operation. In fed-batch mode, cells are initially grown in a relatively lean production medium followed by regular feed supplementation(s), with feed volumes and frequency of addition optimized to maximize culture productivity over a time course that is compatible with production process constraints. In perfusion applications, cells are initially grown to a targeted cell density, after which fresh medium is added to the growing cell culture at the same rate that conditioned medium is removed; this typically employs a cell retention device to prevent the removal of cells from the production vessel.

The Cellvento® CHO media platform is a series of non-animal origin chemically defined cell culture media and feeds designed for use in either batch, perfusion or fed-batch CHO cell-based bioproduction. They were designed for use with CHO-S, CHO-DHFR- and CHO-K1 mammalian cell expression systems. Specifically, in fed-batch mode, the Cellvento® CHO series of media and feeds was developed to enhance, sustain and extend typical CHO cell cultures by augmenting media components (such as amino acids and vitamins) typically consumed during customary fed-batch processes. Maximizing cell growth and culture duration typically yield improved cellular productivity, thereby generating maximal recombinant protein/monoclonal antibody (mAb) yield.

The Cellvento® CHO-200 media series consists of production media, specifically designed to support initial cell growth along with a companion feed containing a proprietary formulation of amino acids, vitamins, trace elements and other components that enhance cellular productivity during biotherapeutic manufacturing. A cysteine/tyrosine feed supplement is also provided in order to maximize feed process flexibility and to provide opportunities for fed-batch process optimization during process development. In addition, while glucose is present in some Cellvento® feed formulations, its level should be monitored and adjusted as needed. **Figure 2** shows the Cellvento® CHO media platform and the individual media and feeds, for the three predominant CHO cell lines commonly used in mammalian cell bioprocessing.

**Figure 2**.

Cellvento® CHO Media Platform for CHO Cell-Based Bioprocessing.

| Application/Cell line | CHO-S | CHO-DHFR- & slow growing cell lines | CHO-K1 |
|---|---|---|---|
| Production Medium & Companion Feeds in Fed-Batch | Cellvento® CHO-200 | Cellvento® CHO-210 | Cellvento® CHO-220 |
| | Cellvento® Feed-200 | Cellvento® Feed-210 | Cellvento® Feed-220 |
| | Cys/Tyr Separate Feed | Cys/Tyr Separate Feed | Cys/Tyr Separate Feed |
| Dry Powder and Liquid Media for all Cellvento® CHO Media | | | |

This document serves to provide guidance for the preparation and filtration of media and feeds in the Cellvento® CHO media platform, as well as best practices for adaptation of cells to Cellvento® CHO media from various background media formulations. Whereas each Cellvento® CHO product includes a general range of recommended conditions for evaluation, this paper provides more specific guidance for media and feed screening, and troubleshooting suggestions for cell lines and medium/feed combinations that do not perform to expected outcomes.

# Media, Feed and Cell Line Preparation for Cell Culture Process Development

## A. Media Reconstitution Recommendations

Most commercially available cell culture media are available as liquids for small scale performance evaluations, though larger media volumes usually require reconstitution from dry powder formulations. There are several important considerations for reconstituting cell culture media from powders, especially at scales greater than 4 L (where use of a stirring plate and magnetic bar are less feasible). Briefly:

- Floating media powders can be challenging to solubilize; generation of rigorous mixing, and mixing for at least 60 minutes is recommended for effective reconstitution.
- In order to generate rigorous vortex, dry powder media should be initially reconstituted into water representing no more than 40% of the final media volume (this generates greater mixing power than that possible with larger initial mixing volumes); supplementation and pH adjustment should be completed after reconstitution but prior to dilution to the final volume.
- Most Cellvento® CHO media lack glutamine, hypoxanthine and thymidine, in order to provide flexibility with selection of the cell line; media may be supplemented as shown in **Figure 3** below, according to the cell line used for recombinant protein generation (refer to media product insert for specific supplementation instructions).
- Cellvento® CHO-220 medium contains hypoxanthine and thymidine (HT), and is specifically designed for use in CHO cell-based mammalian expression systems not utilizing the DHFR selection method.

**Figure 3.**

Supplementation Recommendations for CHO Cell Growth and Production in Cellvento® CHO Cell Culture Media.

| | Cell type | Expression system | Glutamine | HT | Selection |
|---|---|---|---|---|---|
| CHO S | Parental | - | + | + | - |
| | Recombinant | GS system | - | + | |
| | | antibiotic resistance | + | + | antibiotic |
| | | DHFR | + | *- | MTX |
| CHO K1 | Parental | - | + | + | - |
| | Recombinant | GS system | - | + | MSX |
| | | antibiotic resistance | + | + | antibiotic |
| | | DHFR | + | *- | MTX |
| CHO DG44 | Parental | - | + | + | - |
| | Recombinant | antibiotic resistance | + | - | antibiotic |
| | | DHFR | + | *- | MTX |

*The production (N) reactor may be supplemented with HT to fine-tune production.

**Figure 4.**

Filter Recommendations for Sterile Filtration of 50 –1000 L Batch Sizes of Cellvento® CHO Cell Culture Media Using 0.1 µm Sterilizing Grade Filter with Mycoplasma Protection and 0.2 µm Sterilizing Grade Filters.

| | | Recommended Filter | |
|---|---|---|---|
| Batch Size [L] | Filtration Time [min] | 0.1 µm sterile filtration w/mycoplasma reduction using Millipore Express® SHR w/onboard prefilter | 0.2 µm sterile filtration using Millipore Express® SHF |
| 10 | 10 | Opticap® XL300 | Opticap® XL150 |
| 50 | 15 | Opticap® XL600 | Opticap® XL300 |
| 100 | 20 | Opticap® XL3 | Opticap® XL600 |
| 200 | 30 | Opticap® XL5 | Opticap® XL600 |
| 500 | 60 | Opticap® XL5 | Opticap® XL3 |
| 1000 | 120 | Opticap® XL5 | Opticap® XL3 |
| 2000 | 180 | Opticap® XLT10 | Opticap® XL5 |

- After reconstitution, Cellvento® CHO media should be sterile-filtered according to recommendations shown in **Figure 4**.
- After reconstitution and sterile filtration, specific Cellvento® CHO media formulations should be stored according to individual media product recommendations; generally, all media should be stored at 2-8°C and protected from prolonged exposure to light.

## B. CHO Cell Cryovial Reconstitution Recommendations

In reconstituting CHO cells from a frozen cryovial, the following steps are of particular importance to successfully recover cells for expansion:

- Thaw cells rapidly in a 37°C water bath, and transfer the cells into fresh, pre-warmed media as soon as feasible
  - In some cases, slow, drop-wise addition of thawed cryovial contents into fresh media can minimize susceptibility of cells to osmotic shock.
- Determine how best to rapidly remove residual freezing media (often contains dimethyl sulfoxide, which is toxic to cells)
  - Cells can be diluted into fresh media, centrifuged at low speed (i.e. 200-300 g, 10 min) and reconstituted into fresh media.
  - Cells can be diluted into fresh media, and passaged 24 hours post-thaw into fresh media to further dilute freezing media (this technique is more commonly used for cells susceptible to damage by centrifugation).
- Seed cells at relatively high cell densities (i.e. 0.5–1.0x$10^6$ cells/mL), and passage at frequencies which ensure that the cells are growing in mid-logarithmic phase at time of passage.

## C. Cell and Media Adaptation Recommendations

Two common techniques are typically utilized to adapt cells from an original, to a “follow-on” media formulation. For cells that are transferred between two media formulations with similar properties, direct transfer to the new media is often employed. However, for cells transferred between two significantly different media formulations (i.e. from serum-containing to serum-free chemically defined media formulations), more gentle techniques are often required. CHO cells may need to be progressively and deliberately adapted to new media formulations.

**Figure 5**.

Suggested Progressive Cell Culture Media Adaptation Approach for CHO Cells.

| Ratio of current medium vs. new medium (in %) | Seeding Density ($x10^5$ cells/mL) | Evaluation of Cell Growth | Acceptance Criteria for Next Step |
|---|---|---|---|
| 75:25 | ≥ 5.0 | Cell Density, Viability in mid-log growth phase | Normal cell doubling time; VCD > 90% over at least 2 passages |
| 50:50 | ≥ 5.0 | Cell Density, Viability in mid-log growth phase | Normal cell doubling time; VCD > 90% over at least 2 passages |
| 25:75 | ≥ 5.0 | Cell Density, Viability in mid-log growth phase | Normal cell doubling time; VCD > 90% over at least 2 passages |
| 0:100 | ≥ 5.0 | Cell Density, Viability in mid-log growth phase | Adaptation complete when cells maintain normal doubling time; VCD > 90% over at least 2 passages |

While progressive weaning of CHO cells into new media is often accomplished using the approach described in **Figure 5**, some additional adaptation activities can be implemented to improve the probability of technical success. The following adaptation approaches may enhance adaptation success rates for cell lines unresponsive to direct, or typical progressive adaptation to new media:

- Add recombinant albumin (CellPrime® rAlbumin, Cat. No. 9501–50), as a stabilizing protein, to enhance cell growth while cells adapt to new media formulation (recommend evaluation of 1–5 g/L supplementation of cell culture media).
- Ensure that the cells are actively growing in mid-logarithmic phase and that the cells are passaged while cells are still growing.
- Maintain cell cultures at higher cell densities (i.e. ≥ 5.0 x $10^6$ cells/mL).
- Passage cells at least three times in each media condition, and avoid further adaptation until the cell growth rate is consistently in its expected range.
- Adapt cells at more modest increments (i.e. 10–15% versus 25% as shown in **Figure 4**) to reduce stress of adaptation conditions.
- For cells being removed from serum, perform initial adaptation steps in static culture; introduction of agitation (for suspension cultures) to adaptation protocol simultaneously may be too stressful to ensure effective cell adaptation.

## D. Master and Working Cell Bank Generation Recommendations

Adaptation of CHO cells to new media formulations can be a lengthy process, so the generation of working cell banks of intermediate phases of the adaptation process are sometimes valuable to investigators. Generation of intermediate working cell banks can help mitigate the risk of project delays from the cells failing to adapt effectively to a new media formulation. A general protocol for cell bank generation is as follows:

- Cultures should be in logarithmic growth phase and at least 90% viable for cryopreservation.
- Prepare the freezing medium by combining the current media formulation with 10% (v/v) dimethyl sulfoxide (DMSO). Store freezing media at 2–8°C until use.
- Concentrate CHO cells by centrifugation for 5 minutes at 200–300 g.
- Decant supernatant, and resuspend cells in cold freezing medium at a concentration of at least 1 x $10^7$ viable cells/mL.
- Transfer aliquots of the cell suspension into sterile cryovials.
- Freeze cells at -80°C for 24 hours (ideally using a controlled rate freezer) and then transfer to a freezer capable of maintaining a temperature below -130°C, for long-term storage.

## Media and Feed Screening Recommendations

Ultimately, the goal in the upstream biomanufacturing space is to consistently produce high quality recombinant products in an efficient and cost-effective manner. This goal is achieved, in part, by optimizing the nutrition of the CHO cells used to generate the product of interest. The fed-batch mode of upstream bioprocessing is most commonly used, and relies on an optimized feeding regimen in order to maximize both cell culture duration and productivity. In addition, the timing of feeds is an important consideration to balance energy that cells exert for reproduction versus that used to produce the desired therapeutic product. In other words, balancing growth and production contributes significantly to optimizing efficient recombinant product generation.

The Cellvento® CHO media platform includes media and feeds designed for use in the fed-batch mode of operation. Specifically, they include a production medium, a main feed and a separate cysteine/tyrosine feed. Cysteine and tyrosine are notoriously difficult to solubilize and are added as concentrated, high alkalinity feeds. Adding these components separately provides greater process optimization flexibility during feed development activities. In addition, glucose is also typically added as a separate feed in order to optimize cell growth and productivity.

Beyond optimizing production media and feed formulations, an important consideration in developing a fed-batch process for biomanufacturing is the methodology employed to deliver the feeds. Whereas it is clear that under-feeding a growing cell culture has a negative impact on its performance, a process that over-feeds a culture can likewise have deleterious effects. The amount of feed, as well as the frequency of administration, are important considerations when attempting to devise a robust fed-batch process. **Figure 6** summarizes recommended ranges of feed concentrations and frequencies of administration to be used for fed-batch process optimization experiments. These values are suggested ranges only, and optimal feed concentrations should be determined experimentally.

**Figure 6**.

Recommended Feed Volumes and Frequency Ranges of Cellvento® Feed Products for Fed-Batch CHO-Based Mammalian Cell Processes.

| Parameter | Recommended Range for Evaluation |
|---|---|
| Feed | 1-6% (v/v) |
| Cysteine/Tyrosine Stock Solution | 0.15-0.3% (v/v) of Recommended Stock Solution |
| Glucose | 2-4 g/L (monitor daily and maintain at 4 g/L) |
| Frequency | 24-72 hour feed intervals |

Characterization of optimal feed conditions can be accomplished using various methods, which often depends on the scope of work, availability of equipment and resources to conduct the work, as well as the project timeline. For more modest characterization activities, iterative approaches are often utilized to compare specific feed conditions to a reference (typically a batch culture control). Individual experimental conditions are evaluated empirically, with the best conditions chosen for additional characterization and scale-up. Though effective, this method of evaluation is resource-intensive and may be limited by the number of individual conditions that can be evaluated simultaneously. In addition, because individual conditions are evaluated independently from others, the potential influence of one experimental condition on another may not be detectable using this screening method.

Experimentation by a statistical design of experiments (DOE) approach is another screening method utilized by upstream process development scientists to optimize bioprocess feeding regimens. The advantage of this screening method over an iterative approach is that, if planned appropriately, the experimental output can yield more detailed information (including the elucidation of potential interactions between experimental variables). The challenge with this screening method is that it relies on advanced statistical methods that may necessitate the use of software to interpret the experimental output of large numbers of individual data points. The screening method that is ultimately used in a given process development laboratory is often a product of the level of technical expertise and/or the extent of available resources to complete feeding design studies.

## A Case Study: Feed Optimization DOE for an IgG Expressing CHO-S Cell Line Cultured in Cellvento® CHO-200 Medium

This case study sought to develop an understanding of the feed volume and timing requirements needed to optimize a fed-batch bioprocess using a CHO-S cell line that expressed a proprietary IgG mAb. The experimental approach was undertaken to characterize an optimal fed-batch process, as well as to provide a summary of improvements in cell growth and productivity that were achieved. It was hoped that this study would provide a basis for devising a rationalized fed-batch process for other CHO-based mammalian cell culture bioproduction schemes. The study was conducted in shaker flasks, as this system was well-established in the laboratory. This platform permitted a near simultaneous high-throughput analysis of multiple experimental conditions. Spin tubes, deep well plates and microbioreactors are other suitable platforms with which to conduct media and feed optimization studies.

The purpose of a fed-batch process in cell culture is two-fold; first, a production medium is used to support initial cell growth and production, and secondly, a feed is added to replenish depleted nutrients required for cellular function and to maintain the production phase of the culture. Because the performance of production media and their companion feed(s) are typically interdependent, optimizing a feeding strategy is crucial to achieving a culture that both grows and produces protein effectively. Although recommended ranges of feed volumes and frequencies are typically provided for commercially available media and feeds, optimal feed strategies typically need to be determined empirically for a given cell line and its associated process. **Figure 5** shows the recommended feed volumes and frequencies provided for Cellvento® CHO-200 medium.

In this study, cell growth and production performance were evaluated under conditions where feed composition and/or volumes, as well as frequency of administration, were modified. In addition to the individual contributions these feed factors made to overall cell culture performance, the DOE and analysis also addressed whether these factors interacted with each other to create synergistic effects on overall cell culture performance.

### Materials

1. CHO-S GS cell host expressing a proprietary IgG mAb Cells were adapted into study media for at least 3 passages prior to initiation of experimental evaluations.
2. Cellvento® CHO-200 medium
3. Cellvento® Feed-200
4. Glucose stock solution (400 g/L)
5. Recommended Cysteine/Tyrosine Stock Solution
   I. L-Cysteine hydrochloride monohydrate (CAS 7048-04-6)
   II. L-Tyrosine disodium salt dihydrate (CAS 122666-87-9)

   Preparation:

   a. Measure 0.1 L of Milli-Q® or similar cell culture grade water into an appropriate container and adjust the pH ≥ 13 using 5 M sodium hydroxide.
   b. Slowly add 7.9 g of L-cysteine and 22.45 g of L-tyrosine.
   c. Adjust the pH to 11.3 +/– 0.1 using 5 M sodium hydroxide or 1 M hydrochloric acid and mix for 10-30 minutes to dissolve all components.
   d. Add cell culture grade water to reach a final volume of 0.15 L. Confirm final pH of 11.3 +/– 0.1.
   e. Measure the osmolality of the solution. Final osmolality should be 3,100 +/– 100 mOsmol/kg.
   f. Sterilize by membrane filtration using a 0.22 µm Millipore Express® PLUS or Durapore® membrane filter (bottle cap filter).
   g. Store at 2–8°C protected from light. Reconstituted stock solution is stable for 7–14 days. The stock solution yields concentrations of cysteine and tyrosine of 300 mM and 573 mM respectively, which are subsequently diluted during feeding.
6. Multitron® incubator (Infors AG, Bottwingen, Switzerland); 150 rpm, 25 mm orbital, 80% relative humidity
7. 125 mL shaker flasks (Dow Corning Corporation, MI, USA)
8. ViCell™ XR counter (Beckman Coulter, CA, USA) for viable cell density and viability determinations
9. ForteBio® Octet® system (Pall Corporation, NY, USA) for IgG quantitation

## Methods

The study was established using the conditions described in **Figure 7**. Specifically, CHO-S GS cells expressing a proprietary IgG mAb were grown in shaker flasks where the impact of feed volumes and frequency of feed additions were evaluated using a DOE approach. The feeds evaluated included a main feed, as well as a cysteine/tyrosine feed supplement mixture. The study design consisted of 8 experimental conditions representing all permutations of high and low conditions evaluated for main feed volume, cysteine/tyrosine feed volume, and feed frequency. **Figure 9** describes the specific DOE design conditions employed in this case study. JMP software (SAS Institute, Inc.) was used to design the specific full factorial DOE conditions (**Figure 9**) where the contribution of feed volumes and timing on cell performance could be characterized by statistical modeling.

**Figure 7**.

Operating Parameters and Experimental Conditions Employed to Characterize Optimal Feed Design Characterization Study.

| Experimental Condition | Operating Parameter |
|---|---|
| Culture Type | 125 mL vented cap shaker flask, non-baffled |
| Initial Working Volume | 45 mL |
| Inoculation Density | 3 x $10^5$ cells/mL |
| Agitation Rate | 150 rpm (25 mm orbital) |
| Production Medium | Cellvento® CHO-200 chemically defined culture medium |
| Feed | Cellvento® Feed-200 chemically defined cell culture feed |
| Feed Supplement | Cysteine/Tyrosine Solution |
| Temperature | 37.0 ± 0.5°C |
| Incubator pCO2 | 5% |
| Media pH | 7.0 |
| Harvest Criterion | End culture when viability < 75% |
| Sampling Points | Study days 0, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 |
| Main Feed Volume | DOE Design (see Figure 8) |
| Cys/Tyr Feed Volume | DOE Design (see Figure 8) |
| Feeding Schedule | DOE Design (see Figure 8) |
| Glucose Feed Addition | Daily addition, maintaining levels ≥ 4 g/L |

The first post-inoculation sampling time point is study day 3, followed by daily sampling. Minimal sampling volume (i.e. < 800 µL) is required.

**Figure 8**.

Experimental Design of Experiments (DOE) Outline Utilized to Optimize Feeding Volumes and Timing.

| Experimental Variable | Low Value (-1) | High Value (+1) |
|---|---|---|
| Main Feed Volume | 1% (v/v) | 6% (v/v) |
| Cys/Tyr Feed Volume | 0.1% (v/v) | 0.3% (v/v) |
| Feed Frequency | 72 hours | 48 hours |

**Figure 9.**

Randomized Experimental Setup for DOE Analysis of Feed Optimization. (Individual conditions were evaluated in triplicate). Pattern designation of x,y,z in column 2 refer to cysteine/tyrosine feed, main feed and frequency, respectively.

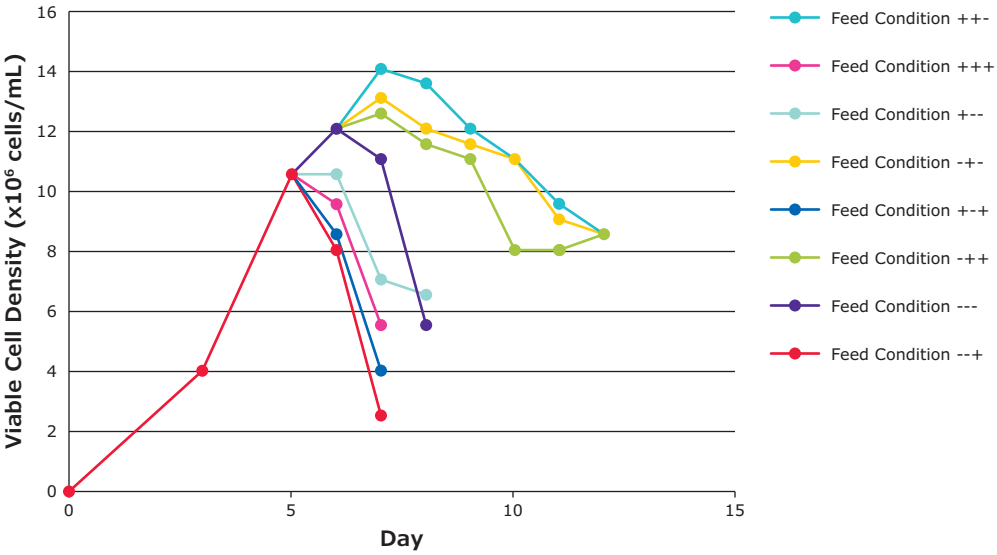
| | Pattern | C/T Volume (x) | Feed Volume (y) | Frequency (z) |
|---|---|---|---|---|
| 1 | −++ | -1 | 1 | 1 |
| 2 | −−− | -1 | -1 | -1 |
| 3 | +++ | 1 | 1 | 1 |
| 4 | −−− | -1 | -1 | -1 |
| 5 | −−+ | -1 | -1 | 1 |
| 6 | +−− | 1 | -1 | -1 |
| 7 | −++ | -1 | 1 | 1 |
| 8 | +++ | 1 | 1 | 1 |
| 9 | −+− | -1 | 1 | -1 |
| 10 | +++ | 1 | 1 | 1 |
| 11 | −−− | -1 | -1 | -1 |
| 12 | −+− | -1 | 1 | -1 |
| 13 | +−− | 1 | -1 | -1 |
| 14 | +−+ | 1 | -1 | 1 |
| 15 | +−− | 1 | -1 | -1 |
| 16 | ++− | 1 | 1 | -1 |
| 17 | +-+ | 1 | -1 | 1 |
| 18 | ++- | 1 | 1 | -1 |
| 19 | +−+ | 1 | -1 | 1 |
| 20 | −+− | -1 | 1 | -1 |
| 21 | ++- | 1 | 1 | -1 |
| 22 | −−+ | -1 | -1 | 1 |
| 23 | −−+ | -1 | -1 | 1 |
| 24 | −++ | -1 | 1 | 1 |

## Results

The study was set up and executed, as described in the Materials and Methods sections. After inoculation, cell counts and antibody titers were determined at regular intervals using a ViCell™ XR counter (Beckman Coulter, CA, USA) and ForteBio® Octet® system (Pall Corporation, NY, USA). Individual conditions were maintained until the cell viability dropped below 50%, at which point the experiment was terminated. All data points shown are the average of triplicate determinations with corresponding error bars representing error of one standard deviation. As shown in **Figure 10**, the cell growth profiles of several experimental conditions showed varying cell growth performance, especially over culture days 7 to 12. Generally, the various growth profiles indicated that with the cell line used in the study, opportunities existed to optimize media and feed performance by varying the feed volumes and/or frequencies of administration.

**Figure 10**.

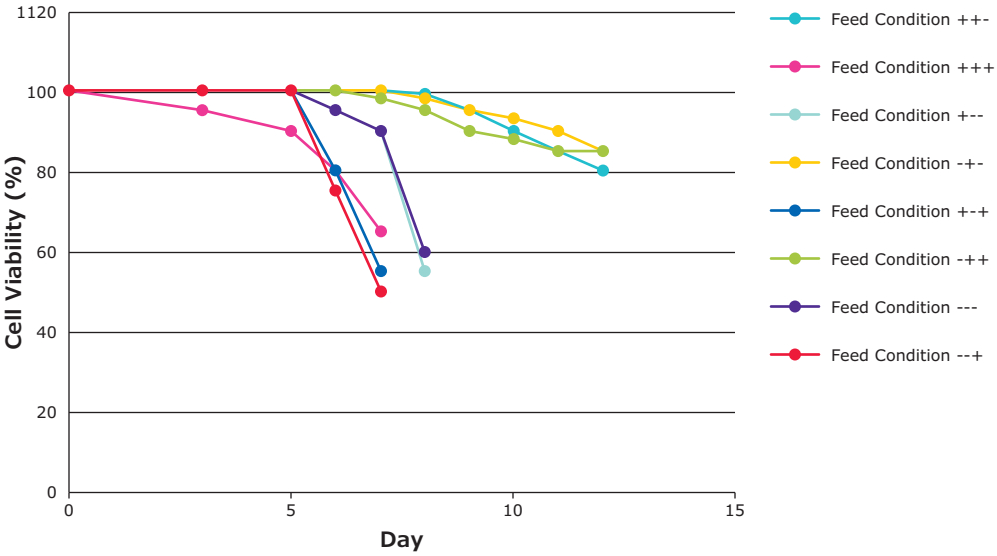
CHO Cell Density Plotted Versus Time. In the legend, feed condition x,y,z represents cysteine/tyrosine feed volume, main feed volume, and frequency of feeding, respectively.




The growth curves in **Figure 10** were essentially clustered into two groupings, where one group had peak cell densities at approximately study day 6, and the other group had peak viable cell densities around study day 8. Statistical analysis of growth curve results was completed using JMP software and is reviewed later in this Results section. Similarly, viability curves were generated for each experimental condition evaluated. **Figure 11** shows cell viability (in percentage) plotted versus culture time. As seen with the growth curves in **Figure 9**, cell viability profiles varied significantly based on the experimental conditions used to feed the CHO-S cells used in this study.

**Figure 11**.

CHO Cell Viability Plotted Versus Time. In the legend, pattern x,y,z represents cysteine/tyrosine feed volume, main feed volume, and frequency of feeding, respectively.

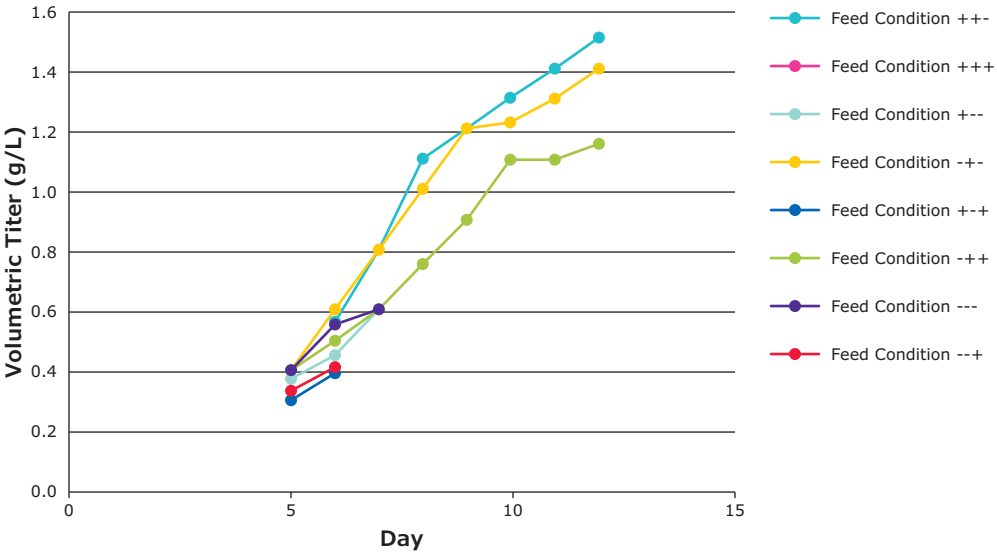



As in **Figure 10**, **Figure 11** had two main groupings of viability curves. The group of curves with prolonged viability generally corresponded to higher, or more frequent feeds. Again, statistical analysis of these viability curves was completed using JMP software, and is reviewed later in this Results section.

**Figure 12** shows the volumetric IgG titer profiles for all experimental conditions evaluated in this study. As observed in the cell growth and viability curves, volumetric titers varied significantly depending on the experimental conditions used. Perhaps not surprisingly, the study conditions yielding the highest titers were also the ones where the highest growth profiles were observed, suggesting that highest product generation was achieved primarily through maximizing biomass in a given experimental system.

**Figure 12**.

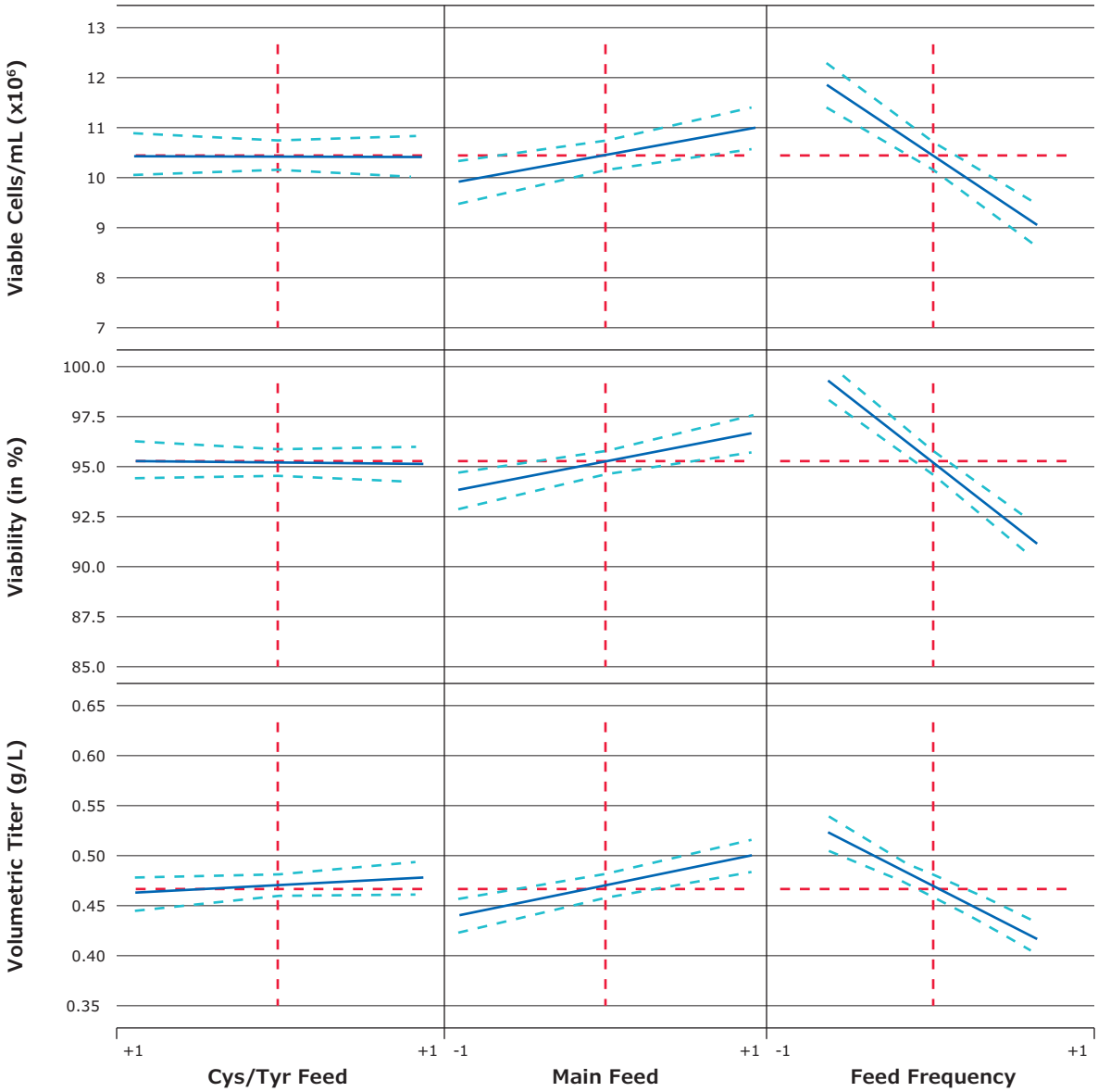
CHO Cell Productivity Plotted Versus Time. In the legend, pattern x,y,z represents cysteine/tyrosine feed volume, main feed volume and frequency of feeding, respectively.



Analyzing the data together, the feed volumes and frequencies had significant effects on cell growth, viability and productivity. Specifically shown in **Figure 13**, higher levels of the main feed significantly improved viable cell density, cell viability, and production of IgG, as did the more frequent 48 hour feed schedule. Interestingly, the evaluated cysteine/tyrosine feed had no impact, positive or negative, on overall cell performance under the conditions that were evaluated (cell growth, viability or productivity).

**Figure 13**.

Prediction Profiler of the Impact of Feed Volumes and Timing on Cell Growth, Viability and Titer. JMP software was used to analyze the results of a designed experiment and statistical evaluation revealed that feed volumes had negligible impact on CHO-S GS cell growth or viability, yet improved cell titer at the higher level evaluated. In addition, the more frequent 48 hour feed schedule consistently enhanced cell growth, viability and productivity.



## Conclusions/Discussion

The results of this case study revealed that for this cell line and media/feed combination, more frequent feeding (i.e. every 48 hours) and using the higher concentration (i.e. 6% v/v) enhanced performance. In this specific scenario, the amount of cysteine/tyrosine feed did not impact the overall cell performance under the conditions tested. It is critical to note, however, that these results should be considered unique to the cell line evaluated, and that the results presented here may not be indicative of what could be achieved with other cell lines and/or media and feed formulations. Only through a thorough media and feed screening approach, similar to that presented here, can optimal media and feed conditions be determined. Development of a fed-batch bioprocess for generation of monoclonal antibodies or recombinant proteins in a mammalian cell culture system requires the use of effective media and feeds, as well as the optimization of a feeding regimen in order to maximize product generation. Significant improvement in cell culture performance was achieved in this study by evaluating the appropriate combination of process inputs; however, complete optimization of this feeding process would likely require additional experimentation to fine-tune the feeding regimen.

This study demonstrates that while Cellvento® CHO-200 medium and Cellvento® Feed-200 designed for use in CHO-S-based mammalian cell culture are effective, their implementation needs to be tailored to each individual CHO cell line. The results of this case study demonstrate the necessity to identify appropriate process conditions in order to improve a fed-batch process. Put another way, the specific data generated in this case study are less important than the general description of the approach used to devise an upstream process evaluation scheme for any given medium and feed regimen.

### Troubleshooting Tips

| Situation | Cause | Action |
|---|---|---|
| Poor growth, productivity | Cells under-fed | Optimize feeding regimen |
| | Cells over-fed | |
| | Cells not adapted to media | Optimize cell/media adaptation |
| | Poor media preparation | Follow media powder reconstitution instructions |
| Slow cell growth | Cells under-fed | Optimize feeding regimen |
| | Low cell seeding density | Characterize optimal cell growth conditions |
| | Poor cell adaptation | |
| | Non-optimal cell passaging | |
| | High medium osmolarity | Confirm appropriate media supplementation |
| | Culture contamination | Initiate new cultures; test cell banks for contamination |
| Good cell growth, poor productivity | Cells over-fed | Optimize feeding regimen |
| | Poor transgene genetic stability | Select higher expressing cell clone(s) |
| | Cell clumping | Supplement with anti-clumping agent |

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**References**

1. Application Note: Perfusion application in Mobius® Single-use Bioreactors. MilliporeSigma Lit. No. AN5372E00; 04/2015.
2. Application Note: Cell Culture Media Filtration. Filter Selection and Sizing. MilliporeSigma Lit. No. AN5144EN Ver.1.0; 4/2020.


The typical technical data above serve to generally characterize the cell culture media in industry-relevant expression systems. The product information is available separately from the website: **MerckMillipore.com**

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|---|---|
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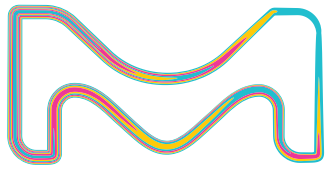




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