Very Rapid Feed Medium Development for Three Different CHO Cell Lines

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Abstract

Rational design of feed media for fed-batch production of therapeutics can be accomplished very rapidly and effectively using spent media analysis and previous experience. Two strategies for feed development have been previously described and are simple replacement and rate dependent replacement of depleted nutrients. Although the rate dependent strategy can result in greater improvements in cell growth and production in fed-batch culture, the simple replacement strategy can be implemented quicker and with more ease. Use of this simple replacement strategy for rapid feed development is shown for three different recombinant CHO cell lines. For all three cell lines, dramatic improvement in cell growth and production was seen. Using this strategy for development of fed-batch culture feed media and processes, improvements in volumetric production ranging from 50 to 200% were seen compared to batch cultures. Feed media developed in this way can be used to rapidly improve culture performance for production of therapeutics during clinical trials and also provide a good starting point for further feed media and process optimization for production for later clinical trials and full scale therapeutic manufacture.

Methods

Sequence of Development

The study was divided into two phases.

First, feed media formulas were developed for three different cell lines using spent media analysis to determine depletion of amino acids and vitamins during batch culture. Other components including metabolites, metabolic intermediates, and lipid precursors were added based on previous experience.

Second, the feed media and feeding strategy were evaluated in shake flask or bioreactor cultures for all three cell lines and compared to batch cultures. In this study, cell growth and volumetric production were evaluated. Cultures were harvested after viability dropped below 50%.

Cell Culture

Three different recombinant CHO cell lines producing IgG antibodies were used in this study. Batch cultures for feed development were carried out in 125mL shaker flasks with 30mL culture media on an orbital shaker platform set at 120RPM in an incubator at 37°C and 5% CO_2. The culture media for each cell line was different – Cell line 1 was cultured in an optimized medium and Cell Lines 2 and 3 were cultured in off-the-shelf media. Cells were seeded at 2-4x10^5 cells/mL and viable cell density was measured daily using a Vi-Cell XR™ cell viability analyzer (Beckman Coulter, USA). Samples for spent media analysis were collected on day 0 and after the day of peak cell density.

Spent Media Analysis

- 20 Amino acids and 7 water soluble vitamins were measured using reverse phase chromatography on an Agilent 1100 HPLC system.
- Volumetric production was determined by HPLC using a protein-G column and UV absorbance detection at 214nm.

Fed-Batch Cell Culture

Culture runs comparing batch with fed-batch process modes were conducted in 125mL shaker flasks or bench scale bioreactors depending on the cell line. The fed batch process involved feeding 5% working volume (wv) on days 3 and 4 for a total of 10% wv of feed.

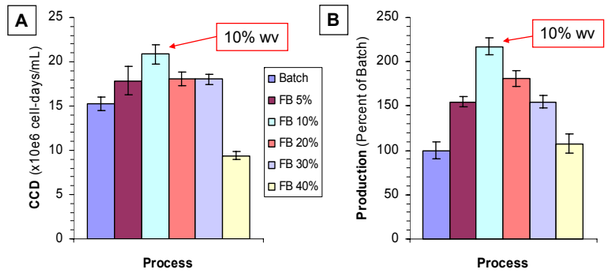
Results

1. Simple Replacement Fed-Batch Cell Culture Strategy

A generic simple replacement fed-batch strategy was previously developed for use in this study. Briefly, it involved development of feeds specific for each cell line based on spent media analysis for amino acids and water soluble vitamins and previous experimental data for other components. The feeds were concentrated to 20x and fed in two bolus feeding events (on culture days 3 and 4). The volume of feed added to cultures was seen to have a significant effect on performance both for cell growth and production and 10% was chosen for use in this study (Figure 1).

1. Simple Replacement Fed-Batch Cell Culture Strategy (cont.)

Figure 1. Effect of Feed Volume on Cell Growth and Production of a Representative Recombinant CHO Cell Line. Panel A shows Cumulative Cell Density (CCD) and Panel B shows Relative Volumetric Production of batch (B) and fed-batch (FB) cultures. Fed-batch cultures were fed on days 3 and 4 with total volumes ranging from 5% to 40% (FB 5% - 40%) of the total working volume (wv).



2. Prototype Feed Media Development

Recombinant CHO cells were grown in 125mL shaker flasks and samples were taken after the day of peak viable cell density for spent media analysis. To determine depletion, concentrations of amino acids and vitamins in these samples were compared with fresh media (Figures 2 – 4).

Figure 2. Rapid Custom Feed Media and Process Development Leveraging Previous Experience

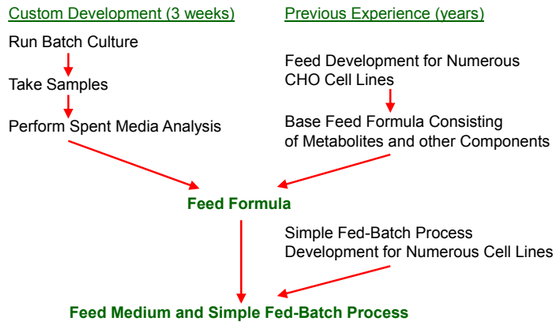
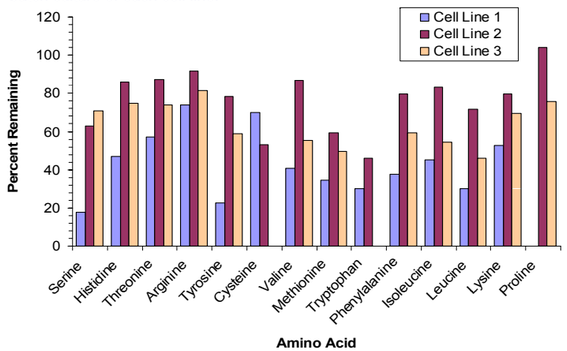
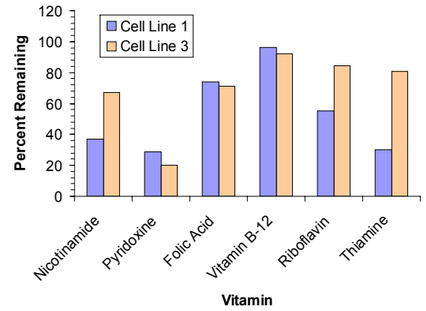


Figure 3. Depletion of Amino Acids During Batch Culture. Data for 14 amino acids is shown. Depletion was determined by calculating the percent of each amino acid remaining after the day of peak of cell density during batch culture of each cell line.



2. Prototype Feed Media Development (cont.)

Figure 4. Depletion of Water Soluble Vitamins During Batch Culture. Data for 6 vitamins is shown for Cell Lines 1 and 3. Depletion was determined by calculating the percent of each vitamin remaining after the day of peak of cell density during batch culture of each cell line.



3. Evaluation of Prototype Feed Media

Figure 5. Growth of Cell Line 1 in Batch and Fed-Batch Cultures.

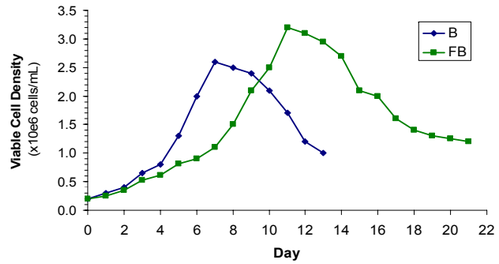


Figure 6. Growth of Cell Line 2 in Batch and Fed-Batch Cultures.

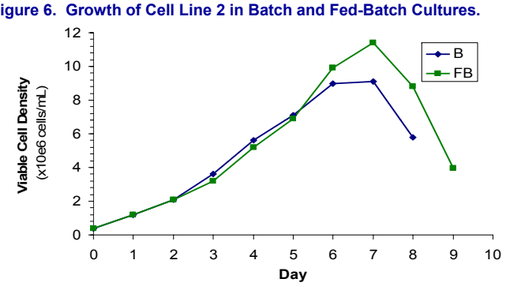
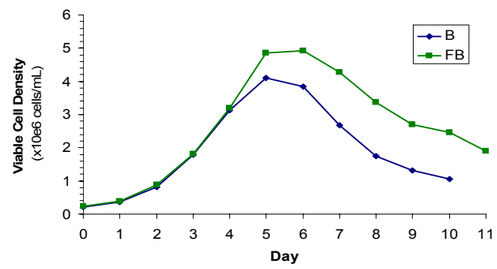


Figure 7. Growth of Cell Line 3 in Batch and Fed-Batch Cultures.



3. Evaluation of Prototype Feed Media (cont.)

To evaluate the effectiveness of the rapidly developed feed media and simple fed-batch process, batch and fed-batch cultures were compared for each cell line (Figures 6 – 8). Cumulative cell densities were determined by summing the daily viable cell densities. Production was determined relative to that in batch culture for each cell line (Figures 9 – 10, Table 2). Fed-batch culture surpassed batch culture for each cell line tested in both cell growth and production.

Figure 9. Cumulative Cell Density of Batch and Fed-Batch Cultures for Three Recombinant CHO Cell Lines.

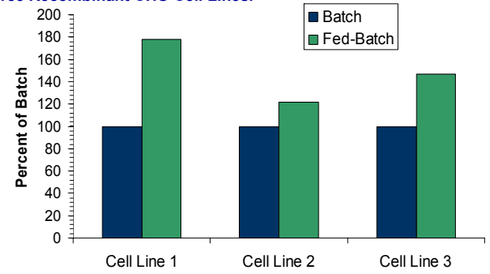


Figure 10. Relative Volumetric Production of Batch and Fed-Batch Cultures for Three Recombinant CHO Cell Lines.

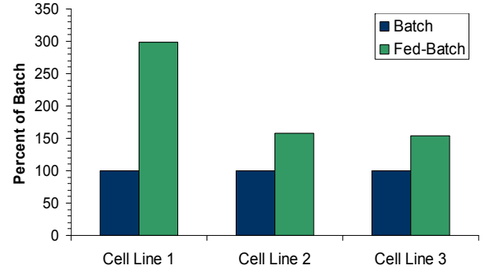


Table 2. Improvements in Fed-Batch Culture using Rapidly Designed Feed Media and a Simple Fed-Batch Process.

Parameter	Percent Improvement of Fed-Batch Cultures		
	Cell Line 1	Cell Line 2	Cell Line 3
Cumulative Cell Density	+78%	+22%	+47%
Volumetric Production	+199%	+58%	+54%

Summary & Conclusions

1. **Effective chemically-defined feed media were developed in about 3 weeks each for 3 CHO cell lines.**
2. **The feed media developed for the cell lines were significantly different.**
3. **The feed media prototypes were used with an easy to implement fed-batch culture process.**
4. **The product yield of fed-batch cultures using the prototype feed media were increased by 54 – 200%.**
5. **The feed media prototypes as shown here can be used as a starting point for further media and process development.**

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