Rapid Chemically-Defined Feed Media Development to Improve Simulated Cell Culture Processes

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Abstract

Media development is an integral step in optimizing a cell culture process. Modification to the growth medium in an existing process may not be a realistic option due to various constraints. Implementing an optimized feed medium may be the most effective alternative means of improving culture performance, whether starting from a batch or a fed-batch process. To challenge this approach, feed media were developed to complement growth media with unknown formulations in simulated cell culture processes. The simulated processes consisted of two commercially available growth media paired with two CHO cell lines. This case study details a two month, four phase plan to design chemically-defined feed media to improve a culture process. The four development phases were: feed media survey, component screen/optimization, amino acid balancing, and formula verification. In both cases, a three-fold increase in antibody concentration over batch was achieved. In addition, superior performance of the resulting feed medium was observed compared to five commercially available feeds. This case study highlights the potential benefit of feed development for a given cell culture process, within a condensed timeline.

Materials & Methods

Cell culture was performed in 125mL Erlenmeyer flasks with a 30mL initial working volume. Cultures were maintained at 37°C, 5% CO_2, and agitated at 120rpm on an orbital shaker. Experiments were inoculated at $3x10^5$ cells/mL and counted daily. Fed-batch conditions were fed 30% (of initial volume) during growth phase. 6g/L glucose was fed to all cultures when glucose fell below 2g/L. Cell counts were determined using a Vi-Cell XR Cell Viability Analyzer (Beckman Coulter). Glucose levels were monitored using a BioProfile FLEX (Nova Biomedical). Titer was quantified using a protein-A, affinity column (Applied Biosystems). Amino acids were measured using reverse phase chromatography on an Agilent 1100 HPLC system.

	Process I	Process II
Host Cell Line	CHO-M	CHO-S
Growth Medium	EX-CELL ™ CD CHO FUSION	CD OptiCHO™

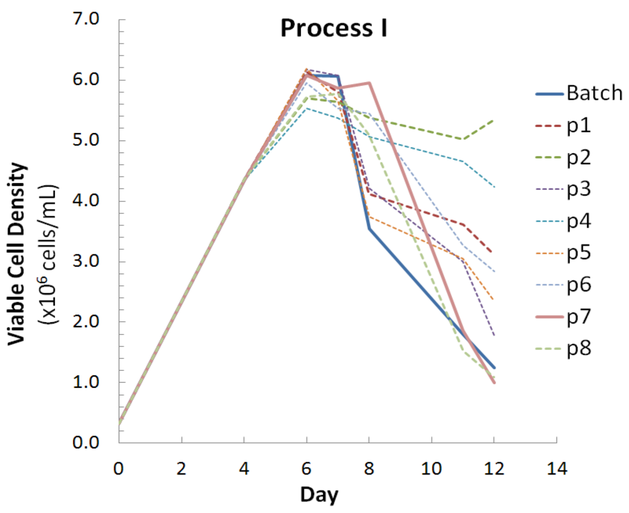
Simulated Cell Culture Process: Two commercially available growth media were chosen in order to simulate a situation in which a growth medium is "locked-in" to an existing process.

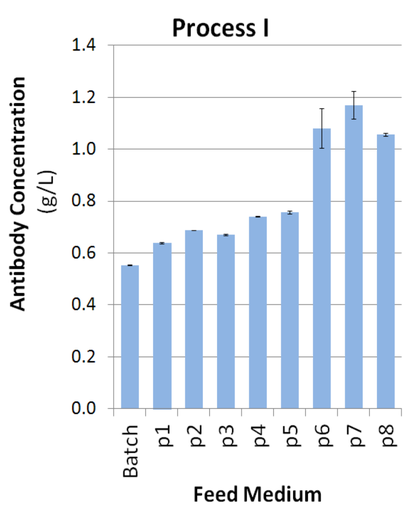
I. Feed Survey → II. Component Screen → III. Amino Acids Balancing → IV. Evaluation

Rapid Feed Media Design: Feed media were designed for each process over a two month period. The resulting feed, BalanCD™ CHO Feed 3, worked well with both processes.

Results

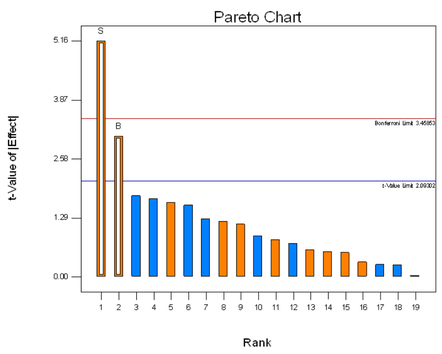
I. Feed Media Survey (Process I)





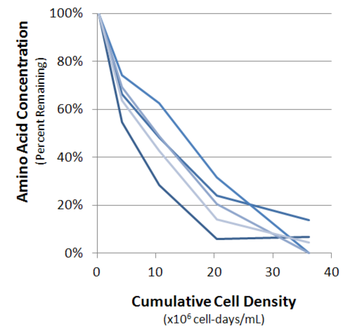
- Survey of eight prototype feed media (p1-p8) from internal library
- Feed Prototype 7 (p7) resulted in a two-fold increase in titer over batch culture, demonstrating the value of having a strong, diverse library
- Feed P7 will be the starting point for further development

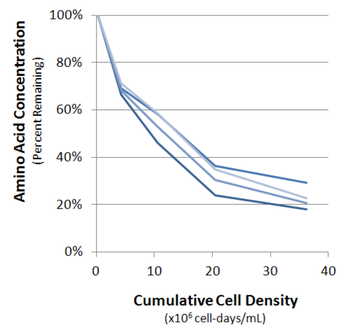
II. Component screen and optimization (Process I)

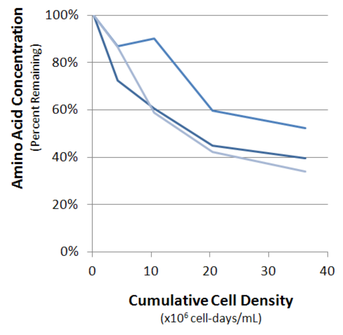


- All feed components except for amino acids and salts were screened using Plackett-Burman design
- Component 'S' and 'B' were identified as critical components based on pareto analysis
- Dose-response experiments were conducted to determine the optimal concentrations of S and B.

III. Amino Acids Balancing (Process I): Amino acids analysis was performed on spent media samples from both batch processes.







Group I

High cell specific depletion rates

- Less than 20% remaining at the end of culture
- Optimized individually and as a group

Group II

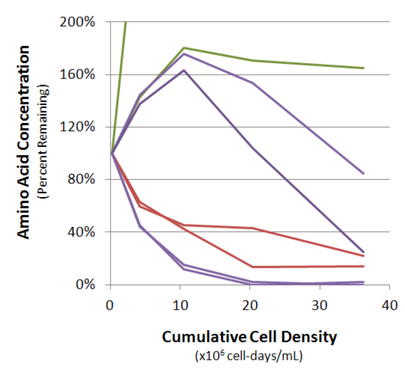
Intermediate cell specific depletion rates

- Optimized as a group

Group III

Low cell specific depletion rates

- Greater than 30% remaining at the end of culture
- Optimized as a group

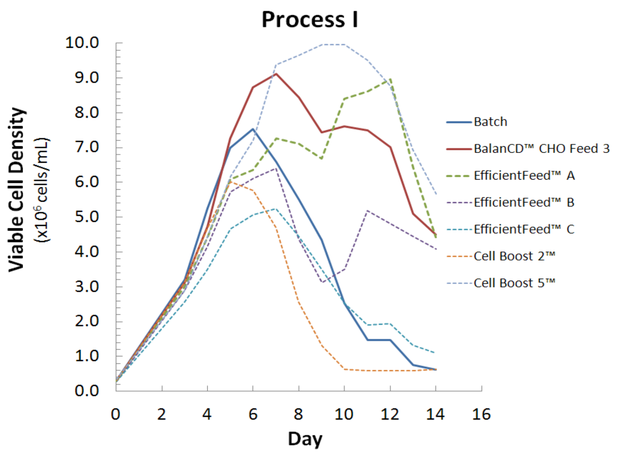


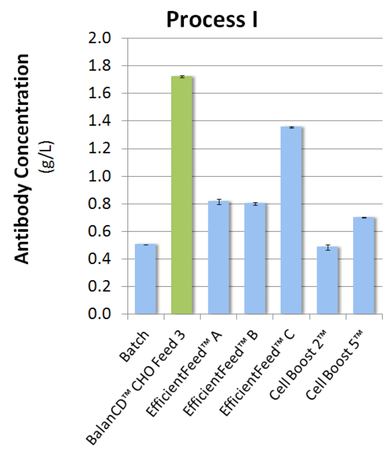
Group IV

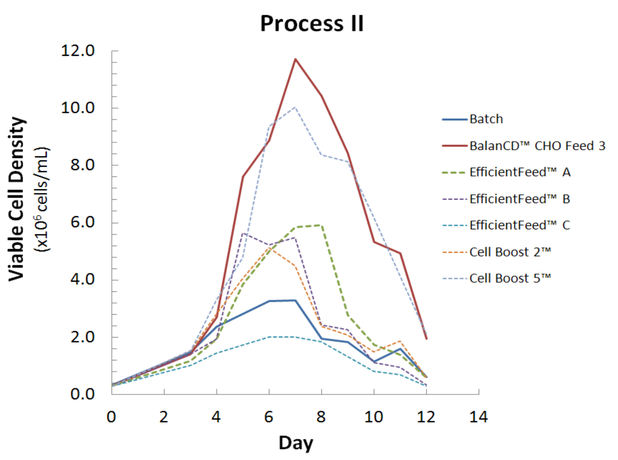
Independent of cell specific depletion rates

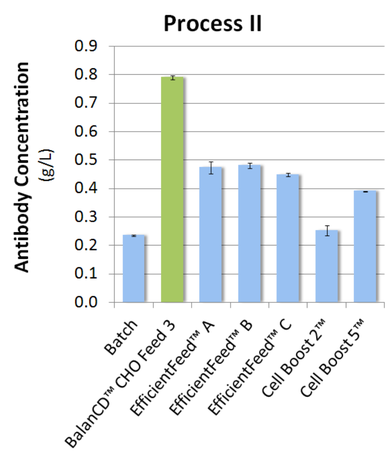
- *Purple* - optimized individually to promote efficient metabolism
- *Red* - maximized concentration to its solubility limit
- *Green* - excluded from feed formulation

IV. Evaluation of BalanCD™ CHO Feed 3 vs. Commercially Available Feed Media: All feed media were supplemented at 10% of culture volume per day on days 1, 3, and 5.









Conclusions

1. This case study details a two month, four phase project to design chemically-defined feed media that improved two simulated batch processes.
2. A three-fold increase in titer over batch in both processes was achieved through rapid feed media design.
3. The resulting feed, BalanCD™ CHO Feed 3, was superior to that of five commercially available feed media.