Very Rapid Development Of A Chemically-Defined Feed Medium For A Specific Cell Line Using Spent Media Analysis – A Case Study

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Abstract

Fed-batch culture processes are widely used for production of recombinant proteins. One of the key elements of developing a fed-batch process is optimization of the feed medium. It can take a significant period of time to fully optimize a feed medium for a specific cell line and that is often frustrated by time constraints for medium and process development. In this case study, an effective chemically-defined feed medium was developed for a recombinant CHO cell line in 3 weeks. This cell line was grown in small-scale batch culture and spent media samples were collected daily that were analyzed for amino acids and vitamins. The specific consumption rate of each component was determined and normalized by the specific uptake rate of glucose. Based on these rates, a feed medium was derived with appropriate concentrations of amino acids and vitamins. Additional nutrients including trace metals were also included in the feed medium. Compared to the batch culture, there was a significant increase in both cell growth and protein productivity. Although the feed medium was not fully optimized, a significantly improved product yield was achieved in a very short period of time using spent media analysis.

Methods

Cell Culture

A CHO cell line producing a recombinant IgG antibody was used in this study. Cultures were carried out in 125mL or 500mL shaker flasks on an orbital shaker platform in an incubator at 37°C and 5% CO_2. Cells were seeded at $2x10^5$ 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 daily.

Spent Media Analysis

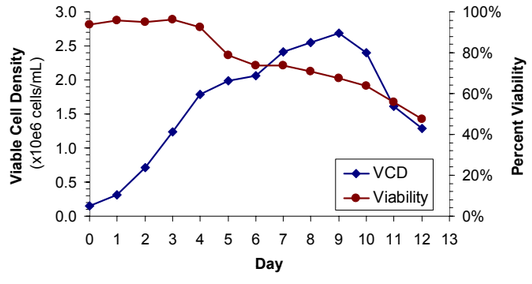
- Glucose, lactate, glutamine, glutamate, and ammonia were measured using a Nova Biomedical BioProfile 400 analyzer.
- 20 Amino acid and 7 water soluble vitamins were measured using reverse phase chromatography on an Agilent 1100 HPLC system.
- Volumetric production was determined by HPLC using a protein-G column and UV absorbance detection at 214nm.

Results

1. Cell Growth in Batch Culture

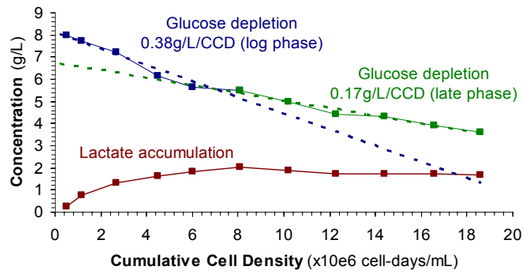
Recombinant CHO cells were grown in 500mL shaker flasks and samples were taken daily for spent media analysis (Figure 1A). Glucose depletion rate was determined by normalizing glucose depletion to cell growth in units of cumulative cell density (CCD; 1e6 cell-days/mL). The rate appeared to have two distinct phases during culture that shifted from 0.38g Glucose/L/CCD in the log growth phase to 0.17g Glucose/L/CCD later in culture (Figure 1B). Uptake rate during log growth was used to derive the feed formula.

Figure 1A. Growth of Model Recombinant CHO Cell Line in Batch Culture.



1. Cell Growth in Batch Culture (continued)

Figure 1B. Glucose Depletion and Lactate Accumulation during Batch Culture.



2. Development of Two Different Feed Media Using Spent Media Analysis

Two methods were employed to develop feed media. Feed Medium 1 (FM1) was designed using a simple replacement strategy based on the depletion of each component on culture day 8. Feed medium 2 (FM2) was designed based on the depletion rates of the measured amino acids and vitamins using a rate dependent replacement strategy. For the rate dependent strategy employed by FM2, the depletion rate of each component during log growth was determined as a function of cumulative cell density (Figures 2A & 2B). Other nutrients were added in both feed media based on previous experience (data not shown). The composition and use of the two Feed Media were significantly different using these two strategies (Tables 1 & 2).

Figure 2A. Amino Acid Depletion During Batch Culture.
Asparagine and proline are shown as examples. Some amino acids appeared to have a single depletion rate over the entire culture, while the others appeared to have a shift in depletion during culture similar to glucose.

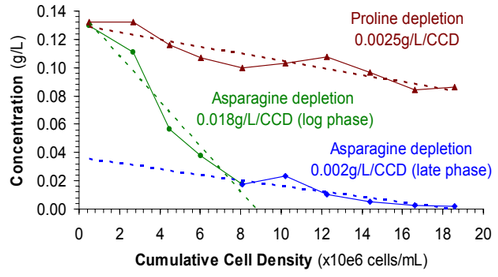
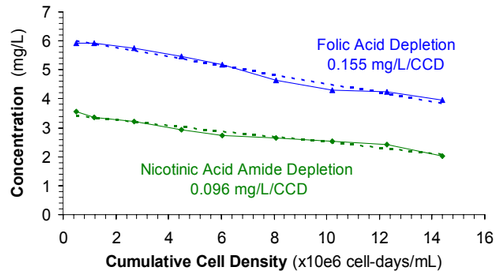


Figure 2B. Vitamin Depletion During Batch Culture.
Folic acid and nicotinic acid amide are shown as examples. The vitamins tested appeared to have a single depletion rate over the entire culture.



2. Development of Two Different Feed Media Using Spent Media Analysis (continued)

Table 1. Comparison of Select Components in Feed Media 1 and 2.

	Feed Medium 1	Feed Medium 2
Nutrient	**g/L**	**g/L**
Arginine	5.20	3.34
Asparagine	3.30	2.13
Histidine	0.75	0.48
Isoleucine	2.50	1.60
Leucine	3.40	2.18
Lysine	2.50	1.60
Methionine	1.00	0.69
Phenylalanine	1.40	0.93
Threonine	1.70	1.12
Tryptophan	0.80	0.52
Tyrosine	1.60	1.05
Valine	2.10	1.35
Cysteine	1.60	1.03
Folic Acid	0.04	0.03
Nicotinic Acid Amide	0.03	0.02
Pyridoxine	0.06	0.04

Each feed medium formula was concentrated as much as possible based on solubility of the components. For FM1 the amount of each component necessary for simple replacement was concentrated approximately 30x. For FM2 the amount of each component depleted per unit of CCD (1e6 cell-days/mL) during log growth was determined and concentrated approximately 120x. FM1 was fed with the fixed amount (2% wv) for 5 days while FM2 was fed based on CCD (0.83% wv/CCD) from days 3 to 9.

Table 2. Comparison of Feed Media 1 and 2 Characteristics.

Parameter	Feed Medium 1	Feed Medium 2
Concentration	~30x	~120x
Schedule	fixed	growth dependent
Volume	10% wv total	0.83% wv / CCD
pH	~7.10	~7.10
Osmolality	538	410

3. Evaluation of Prototype Feed Media

Cells were grown in 125mL shaker flasks with 30mL working volume (n=3). Feed Medium 1 (FB1) was fed at 2% working volume (0.6mL) per day on days 3-7. For Feed Medium 2 (FB2), cell growth in units of CCD (1e6 cell-days/mL) was used to determine the amount of feed added each day (Table 3). Starting from day 3, 0.25mL/CCD was added until day 7; after the peak of cell growth (days 8-9) this was reduced to 0.0625mL/CCD.

The depletion rate based feed method resulted in improved cell growth and production than the simple replacement based feed for this cell line, with volumetric production being increased by approximately 2 fold compared to batch culture (Figures 3 & 4).

Table 3. Feed Schedule and Volumes.

		Feed Volume on Culture Days (mL)							
Process	**Basal (mL)**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Total**
Batch	30								0
Fed Batch 1	27	0.6	0.6	0.6	0.6	0.6			3.0
Fed Batch 2	27	0.45	0.5	0.6	0.7	0.78	0.16	0.16	3.4

3. Evaluation of Prototype Feed Media (continued)

Figure 3. Growth Curve of Batch (B) and Fed-Batch (FB1 & FB2) Cultures.

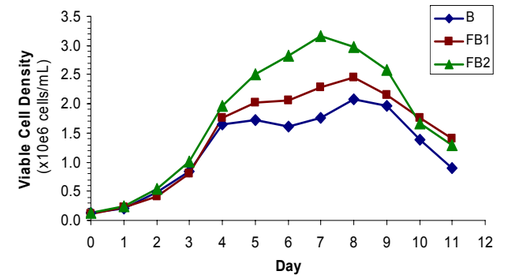
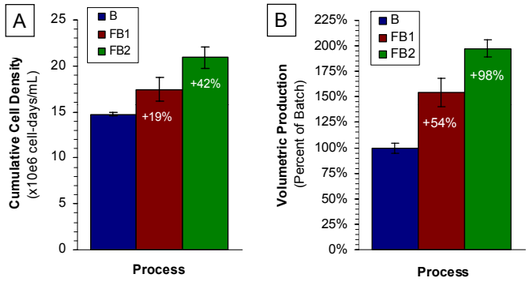


Figure 4. CCD (A) and Volumetric Productivity (B) of CHO cells.



Some Steps for Further Optimization

- Perform Spent Media Analysis on Fed-Batch cultures with Prototype Feed to further refine Feed Medium.
- Evaluate the effect of other media components.
- Use statistically designed experiments to further balance feed components.
- Evaluate the use of hydrolysates.
- Optimize the physical characteristics of feed including concentration.

Conclusions

1. **An effective chemically-defined feed medium was developed for a recombinant CHO cell line in 3 weeks.**
2. **Two different feed media prototypes were derived from the spent media analysis – based on simple replacement and depletion rate based strategies.**
3. **The feed prototype based on the depletion rate strategy led to a greater improvement in performance than the simple replacement based feed.**
4. **The product yield of fed-batch cultures using Feed Medium 2 was increased by 2 fold compared to the batch.**
5. **The feed medium prototypes as shown here can be used as a starting point for further development.**

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