A Rational Method of Integrating Media Development and Fed-Batch Process Strategy: A Case Study

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Abstract

Development of a high performance fed-batch culture process involves development of an optimized set of media and an optimized fed-batch process strategy. Each recombinant cell line is thought to have a unique set of nutritional and process requirements that must be met for maximal performance. Media components for such a process include a set of basal and feed media and any supplements needed. The process strategy determines the methods, amounts, and timing in which each media component is used during culture runs. Process strategy is often developed separately from media optimization although there is believed to be a strong interaction between them. A rational approach to process development involving concurrent development of media and feeding strategy might then lead to greater improvements in culture performance. A case study is presented showing the results of integration of media and feeding strategy development for a recombinant CHO cell line expressing a recombinant protein.

The rational method of integrating media and process development presented here involves several underlying principles. First, since only a six month time frame was given, concurrent development was conducted on multiple parts of the project in order to complete the project in the time allowed. Second, experiments were designed to build upon incremental improvements obtained from the concurrent parts throughout the course of the project. Third, each step of the project was conducted in the context of the current state of development of the fed-batch process strategy. The application of these principles led to integration of media and process development, but also resulted in conflict between the degree of improvement gained at each step with the time required to complete the overall project. In order to maintain a high chance of successfully meeting the project goals within the time frame allowed, flexibility and experience were used to arrive at the best compromises at each step.

The starting point for this project was batch mode culture of a recombinant CHO cell line in an off-the-shelf basal medium with the goal of improving production by 200% in the developed fed-batch process. Over the course of the project, modest increases in culture performance in terms of production were obtained from the individual parts of the project. These increases were approximately 100% for basal optimization, 70% for feed development, and 200% for hydrolysate supplement development. When these components were integrated into an optimized feeding strategy, an increase in production of over 600% was seen, greatly exceeding the project expectations. Data are presented showing the stepwise performance improvements resulting from integration of media developed over the course of the project. These increases in production are shown to be due to both increased cell growth and cell specific production. In this case study, integration of media and process strategy development led to a decrease in the time needed while yielding greater improvements in production of the recombinant protein than those obtained from performing each part separately.

Methods

Cell Culture

The study was conducted using a recombinant CHO cell line that was derived from a DG44 parent cell line and produces an IgG antibody. Cell culture experiments were carried out in 125mL shaker flasks using the following conditions: 30mL working volume, 120 RPM agitation rate, 37ºC, and 5% CO_2. The control basal medium was IS CHO-CD (Cat. No. 91119, Irvine Scientific, USA). Viable cell density was measured daily using a Vi-Cell XR™ cell viability analyzer (Beckman Coulter, USA). Cumulative cell densities (CCD; an estimation of integral of viable cells, IVC) were calculated by summing the daily viable cell densities over the course of each culture. Cultures were terminated once viability dropped below 70% and samples were taken for quantification of production using protein-G affinity chromatography.

Introduction

Project Plan

The study was conducted over a brief six month time frame making careful project management crucial to success. With this time constraint, each media component of the fed-batch culture process was developed concurrently in four overlapping phases (Table 1). The components developed in Phases 1 – 3 included a basal medium, a feed medium, and a hydrolysate supplement.

Phase 4 was organized to incorporate the media components as they were developed and to design a high-performing feeding strategy (Figure 1). As components became available during Phases 1 – 3 they were brought together into an increasingly intricate process strategy resulting in improved culture performance.

Table 1. Project Plan Utilizing Synchronous Development Phases.

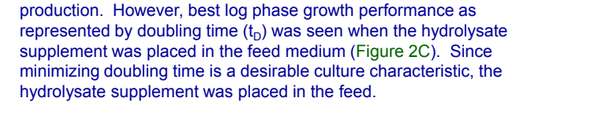


Figure 1. Phase 4: Progressive Integration and Process Improvement.

1. Example of Media Component Integration.

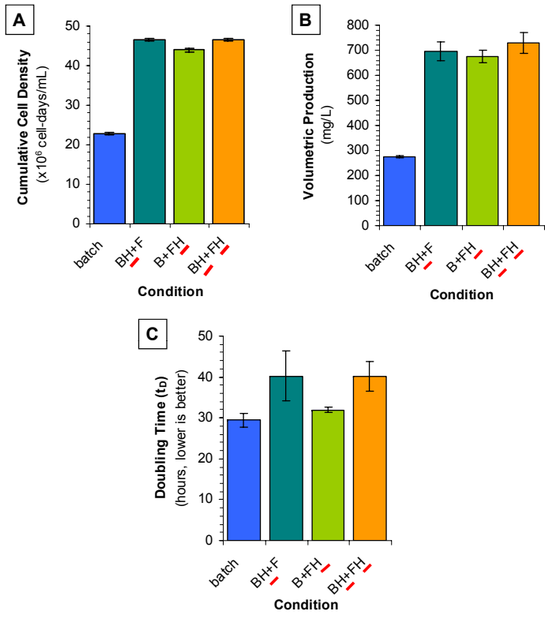
An example of the first part of Phase 4 development was integration of the hydrolysate supplement into the fed-batch process. For simplicity, a single hydrolysate supplement was used. The optimal placement of this hydrolysate was examined by evaluating the cell growth and production performance in cultures with placement in the basal medium, feed medium, or split between both (Table 2 and Figures 2A & 2B). Performance in all three conditions was similar for cell growth and production. However, best log phase growth performance as represented by doubling time (t_D) was seen when the hydrolysate supplement was placed in the feed medium (Figure 2C). Since minimizing doubling time is a desirable culture characteristic, the hydrolysate supplement was placed in the feed.

Table 2. Placement of Hydrolysate Supplement into the Fed-Batch Culture Process.

Condition	Placement	Total Hydrolysate Added
Batch	none	none
BH+F	basal	6g/L in basal
B+FH	feed	6g/L in feed
BH+FH	basal & feed	3g/L in basal, 3g/L in feed

1. Example of Media Component Integration (cont.).

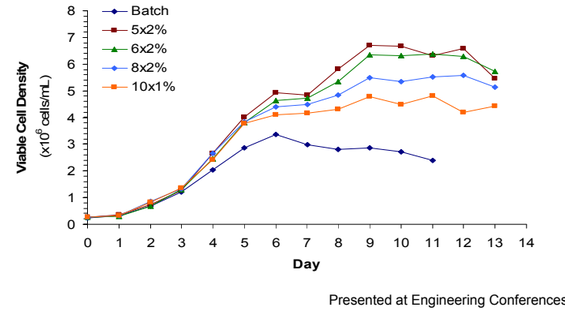
Figure 2. Cell Growth (A), Production (B), and Doubling Time (C) Performance of Cultures with Varying Hydrolysate Placement. The hydrolysate supplement was added to the basal medium (BH+F), feed medium (B+FH), or split evenly between both (BH+FH). The total hydrolysate concentration was 6g/L. Data shown is the average ± SD of three replicates per condition.



2. Example of Process Strategy Development.

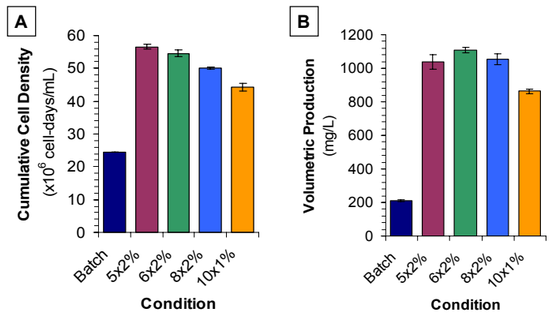
Just as optimization of the feed medium is important to cell growth and production so is optimization of the feeding strategy. For this example, the starting point was a generic strategy with 5 daily feeding events of 2% working volume (wv; total of 10%) starting on day 3 (5x2%). Different feeding volumes and schedules were tested to determine the optimal feeding strategy (Figures 3 & 4). From this experiment and others (data not shown) the optimal feed strategy for this cell line was found to consist of 6 daily feeding events of 2% wv / day for a total of 12% wv starting on day 2.

Figure 3. Cell Growth of Cultures with Different Feed Volumes and Schedules.



2. Example of Process Strategy Development (cont.).

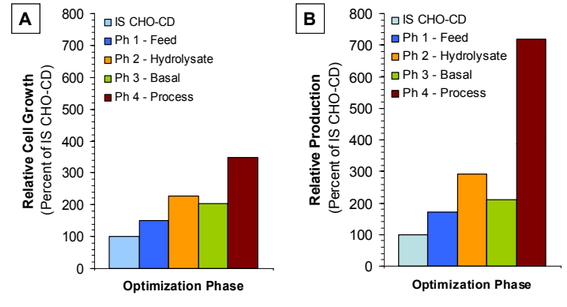
Figure 4. Cumulative Cell Density (A) and Volumetric Production (B) Performance with Different Feed Strategies. Data shown is the average ± SD of three replicates per condition.



3. Summary of Media Integration and Feeding Process Strategy Development.

Phase 4 focused on the integration of the optimized feed, hydrolysate supplement, optimized basal medium and overall fed-batch process strategy development. As individual media components became available during Phases 1 – 3, they were incorporated in Phase 4. Integration of the media components and optimization of the feeding process strategy in Phase 4 resulted in >300% and >600% increases in cell growth and production, respectively, compared to cultures in the starting basal medium (Figure 5).

Figure 5. Relative Cell Growth (A) and Production (B) Performance from Development Phases 1 – 4. Data was taken from verification experiments for each phase and show relative performance compared to control cultures (IS CHO-CD).



Summary & Conclusions

1. **A rational method to simultaneously develop media and process strategy was devised to rapidly optimize culture performance.**
2. **The individually optimized basal medium, feed, and hydrolysate supplement led to 50-300% increases in culture performance.**
3. **Media integration and culture strategy optimization led to improvements of >300% for cell growth & >600% for production.**
4. **This study suggests a strong interaction between media and process strategy in regards to overall culture performance.**

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