

Rapid Enhancement of Antibody Production Through DOE-Based Hydrolysate Supplementation of an Optimized Base Medium

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INTRODUCTION

It has been well documented that different production cell lines have unique nutritional requirements for maximum protein production. With current regulatory standards, a chemically defined medium is the ideal choice. However, undefined components such as serum, BSA, or protein hydrolysates, have historically been eliminated from formulations at the expense of protein production. Additionally, process development timelines and production schedules do not always allow for a complete optimization of a chemically defined medium, feed strategy development, and process development. Therefore, the supplementation of a chemically defined medium with animal free hydrolysates has become a more prevalent and productive industry practice.

Our unique media and process development procedures were utilized to achieve a 4-fold increase in huIgG production from a CHO cell line in less than 18 months. We have previously shown* that an initial 2-fold increase in productivity was achieved through optimizing a chemically defined medium. Here, we have extended that work to include our DOE-based approach to peptone supplementation and process development strategy. A 6-week peptone optimization study resulted in an additional 2-fold increase in antibody production, from 580 ug/ml to 1.2 g/L, in a 14-day shaker flask batch culture. Feed media optimization and strategy development required another 8 weeks and further improved the production level to 1.4 g/L in 14-day shaker flask fed-batch cultures and 1.5 g/L in a bioreactor.

MATERIALS

BD Proprietary Chemically Defined Basal Media (Library Media), Chemically Defined Control Media (Commercially available medium, Medium 15 and revisions), Animal Free Hydrolysates (BD, Phytone™ UF, TC Yeastolate UF, DS100 UF, Yeast Extract UF, and Wheat 2A; Kerry Bio-Science, HyPep 4601), Alamar Blue™ (BioSource), microtiter plates (BD Falcon™), 125 mL shaker flasks (Corning), ELISA Capture plates (BD BioCoat™), pNPP Substrate (Sigma), PBS-Tween 20 (Sigma), anti-hu Ig (G,A,M) (Rockland Immunologics), Protein A HPLC (Waters 2695), ViCell™ Cell Viability Analyzer (Beckman Coulter), NOVA Bioprofile 400 (NOVA Biomedical).

METHODS AND RESULTS

Base Media Optimization

In order to identify an appropriate starting base medium, 33 proprietary chemically defined library media were screened with the CHO cell line in 96-well microtiter plates. During the 6 day culture period, cell proliferation was assayed by Alamar blue on days 0, 3, 4, 5 and 6 (data not shown), and Day 6 IgG production was determined by ELISA (Figure 1). Based on proliferation and production levels, as compared to control medium, multiple media candidates were chosen for further analysis. Cell performance was evaluated in shaker flasks for each of the candidate media over an 11-day batch culture. Cell counts were performed throughout the culture and Day 11 IgG levels were determined by Protein A HPLC (Figure 2). Based on the specific productivity (SPRavg), Medium 15 (M15) was identified as the best base medium candidate for component optimization.

Figure 1: Library Screen for CHO Cell Line Day 6 ELISA Data

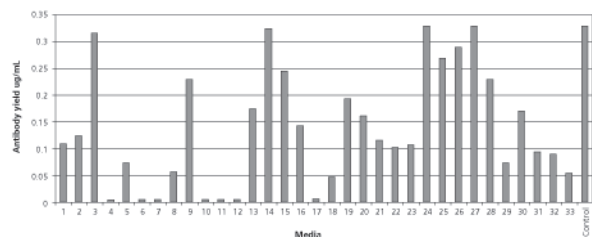
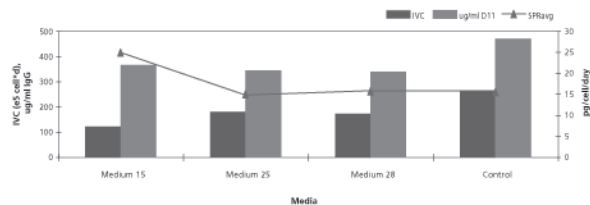


Figure 2: Library Screen For CHO Cell Line Shaker Flask Summary

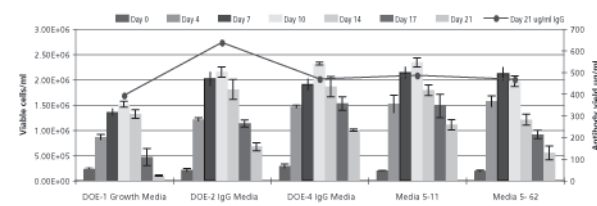


A proprietary mixture design to approximate a response surface design of 5 to 11 media components was used to optimize M15 through several rounds of component optimization. During each round, 5-11 media components were chosen and the resulting 78 media design points were screened with the CHO cells in 96-well microtiter plates for a 7-day culture period. The best media candidates, based on proliferation (Alamar Blue™) and IgG production (ELISA), along with multiple statistical predictions were scaled to shaker flasks for 14-day batch culture performance confirmations. Proliferation was assayed by cell counts and IgG levels were determined by Protein A HPLC.

The proliferation data and Day 7 IgG production data from the 96-well microtiter plate screen for each round of optimization was imported into our statistical package for analysis. Based on proliferation only, Day 7 production only, or the combination of proliferation and Day 7 production, three media formulations were predicted to improve performance (DOE-round X Growth, DOE-round X IgG, and DOE-round X IgG+Growth media, respectively). Along with the 3 statistically predicted media, 2 to 3 media from the plate screen and a negative control were chosen to be scaled to shaker flasks for confirmation of performance as compared to Medium 15 and the proper base media control for each round. Based on the proliferation and IgG production data from the shaker studies, the medium chosen as the best performer was used as the new base for the next round of component optimization.

Following completion of the multiple rounds of component optimization, the final step in determining the optimal chemically defined medium was to conduct a verification shaker study, which included the best performing medium from each round of optimization. Prior to shaker flask seeding, the CHO cells were first adapted through 3 passages in each medium formulation. During the 21-day shaker flask study, glucose levels were monitored and adjusted to maintain concentrations above 2 g/L. This verification study indicated that, following adaptation in each medium formulation, the DOE-2 IgG Medium had one of the greatest effects on cell proliferation and the greatest enhancement of IgG production (Figure 3). (All base optimization data was previously presented in a poster at ESACT 2005 and is available upon request.)

Figure 3: Verification Shaker Study of Top Media Performers from All Optimization Rounds



METHODS AND RESULTS CONTINUED

Peptone Supplementation

Using DOE-2 IgG Media as the new base media, the CHO cell line was screened in a 96-well plate based DOE against 6 animal free peptones and proliferation and production were monitored (Figures 4 and 5). The top 3 performance enhancing peptones at their best concentration were used for a shaker scale up study using another proprietary DOE. Fourteen-day batch cultures were run with multiple time point evaluations of proliferation and Day 14 evaluation of production by Protein A HPLC assay. (Figures 6 and 7) The shaker data results showed that peptone blends 1, 5, 6, and 14 gave 2-fold increases in production as compared to the no peptone feed control. The decision was made to proceed to Feed Strategy Design with Blend 14 as this contained the lowest total g/L of peptone.

Figure 4: Peptone Screen in DOE-2 IgG Medium Proliferation Determined by Alamar Blue

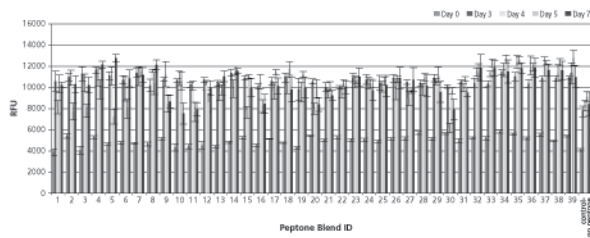


Figure 5: Peptone Screen in DOE-2 IgG Medium Day 7 Production Determined by ELISA

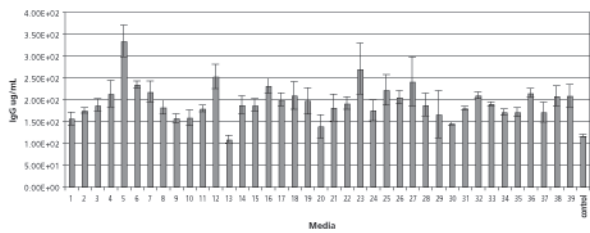


Figure 6: Peptone Optimization in DOE-2 IgG Medium Proliferation Determined by Trypan Blue Exclusion

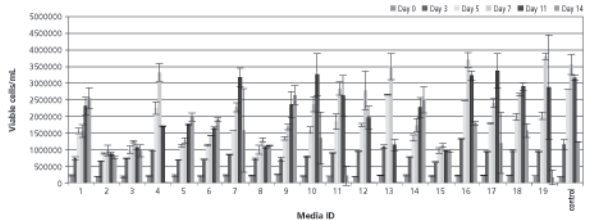
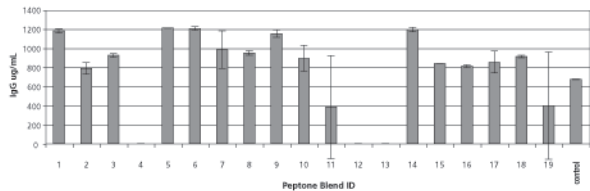


Figure 7: Peptone Optimization in DOE-2 IgG Medium Day 14 Production Determined by Protein A HPLC



Feed Strategy

The CHO cells were cultured for 5 days in a batch culture in DOE 2 IgG Media with peptone Blend 14. On Day 5 of the culture, the cells were aliquoted into multiple shaker flasks and subjected to a proprietary DOE screen of peptones as feed supplements. From the results (Figure 8), peptone Blend 9, consisting of 6 peptones, was chosen as the best combination of starting peptones and feed peptones.

The need to simplify the peptone Blend 9 feed from 6 peptones to 2 peptones was evident and thus tested (Figure 9). This study allowed the feed peptone blend to be reduced to 2 peptones (TC Yeastolate UF and Wheat 2A). The feed timing and a double bolus feed on Days 3 and 7 as compared to a single bolus feed on Day 5 was investigated (Figure 10). Results showed improvements in production by changing to a Day 3 and 7 feed as opposed to a single Day 5 feed.

Results to this point showed an increase in production from 340 mg/L in the original media in a 14-day batch culture to 1.3 g/L production in a 14-day shaker flask fed-batch culture.

Figure 8: Peptone Feed Screen on Day 5 of Culture Day 14 Production Determined by Protein A HPLC

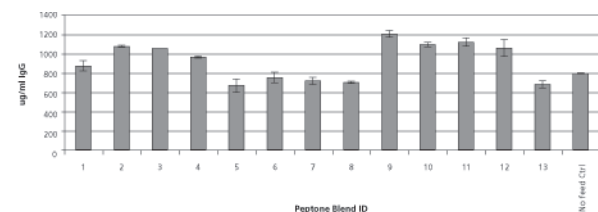


Figure 9: Peptone Feed Simplification Study Production Determined by Protein A HPLC

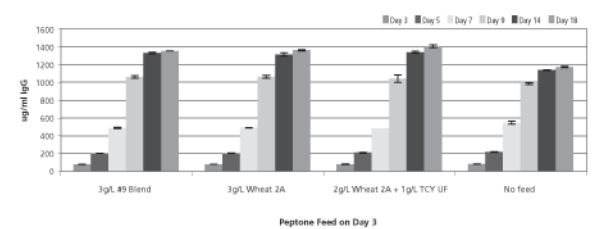
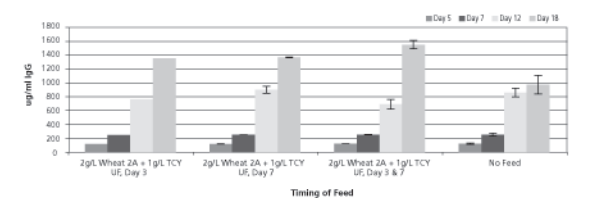


Figure 10: Peptone Feed Timing of Addition Strategy Production Determined by Protein A HPLC



METHODS AND RESULTS CONTINUED

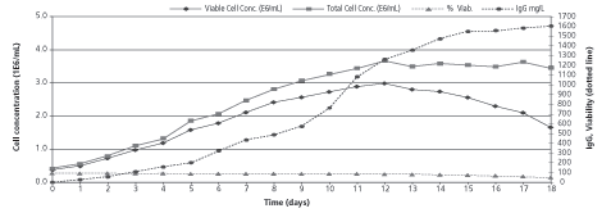
Bioreactor Scale Up

The initial bioreactor study evaluated how the conditions identified during the Feed Strategy development would translate into a stirred tank reactor. Default process control conditions were utilized (dO = 50%, pH = 7.0, agitation = 80 RPM, temperature = 37°C) The vessel was inoculated with 3.7E5 cells/mL into DOE-2 IgG Media with peptone Blend 14. On Days 3 and 7 the cultures were fed TC Yeastolate UF and Wheat 2A to yield 1 and 2 g/L respectively. For the duration of the run, glucose was maintained above 3 g/L.

The run resulted in maximum viable cell density reaching 3E6 cells/mL on Day 12 with viabilities remaining above 75% for a 14 day period. The Day 14 IgG production level was 1.5 g/L with a maximum production of 1.6 g/L reached on Day 18 (Figure 11). This initial bioreactor run showed that the media

and feed conditions are appropriate and scalable for this cell line. Future bioreactor studies will focus on control parameter optimization and additional feed strategies to further increase production in a 14 day fed-batch culture.

Figure 11: Initial Confirmation Bioreactor Run Proliferation and IgG Production



CONCLUSIONS

Base media optimization required 8 months of development time and resulted in an approximate 2-fold increase in production in batch culture shaker system. Initial screening of peptone supplements required 6 weeks and resulted in an additional 2-fold increase in production. Feed strategy optimization using optimized peptone blends and timing improved production in a 14-day fed-batch culture to a final level of 1.5 g/L with initial bioreactor scale up confirming a 1.5 g/L production level in 14 days to demonstrate scalability of the process.