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Process Optimization Using Hydrolysates: Case Studies Demonstrating Increased Performance With Hydrolysate Blends and Feed Strategies

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INTRODUCTION

Successful biopharmaceutical production strategies are dependent upon several key factors that must be optimized for each production process. One of the most important factors in any production process is the selection of the appropriate base medium that is coupled with an effective feed strategy. While chemically defined media and feeds are desired, long development times and high costs cause many companies to look for alternative media optimization strategies. Animal free hydrolysates have been used by these companies to achieve increased production levels with minimal cost and development timeline requirements.

Every hydrolysate is unique due to all of the different factors that contribute to its production. Therefore, it is necessary to test a variety of hydrolysates at a variety of concentrations to ensure that the best supplementation scheme is identified for the particular cell line and base medium. It is also important to identify an appropriate feed strategy to further achieve optimal performance. This can be accomplished by using a feed strategy design where optimal hydrolysate addition times and concentrations are identified. This presentation shows multiple case studies where the optimal hydrolysate supplementation and feed strategies were identified for multiple production cell types, including CHO, hybridoma, PerC6, and NS0.

MATERIALS

CELL LINE

- Three CHO cell lines producing IgG
- Hybridoma cell line producing IgG
- PerC6 cell line producing IgG
- NS0 cell line producing IgG

CULTURE MEDIA AND SUPPLEMENTS

- BD Proprietary Chemically Defined (CD) Basal Media
- Commercially Available CHO Media
- BD Animal Derived Hydrolysate- Protease Peptone #3 (PP3)
- BD Animal-Free Hydrolysates- TC Yeastolate UF (TCY UF), Developmental Wheat Hydrolysate, Select Soytone, Phytone UF, and DS100 UF

EQUIPMENT AND REAGENTS

- 125 mL and 500 mL Shaker Flasks (Corning®) Proliferation – ViCell (Trypan Blue) Production – Protein A HPLC and ELISA IgG

METHODS AND RESULTS

Case Study #1 — Evaluation of Hydrolysate Blends with CHO Line #1 and a Hybridoma Line

The goal of this study was to replace PP3 with an animal free hydrolysate supplementation for both CHO Line #1 and a hybridoma cell line using a commercially available base medium. Both cell lines were screened against a panel of hydrolysates, either individually or as blends, and cell growth and protein production determined. As shown in Figure 1, hydrolysate blends (15, 22, 25, 26, 30) and individual hydrolysates (TCY UF or PP3) enhanced antibody production levels with CHO Line #1. Although the individual TCY UF performed as well as PP3, the animal free hydrolysate blends further increased antibody production with blends 15 and 22 giving approximately 2-fold increases in production as compared to PP3. However, with the hybridoma line not all hydrolysates or blends enhanced antibody production above levels achieved with PP3 (Figure 2). Although this data shows that blend 195 and the individual wheat hydrolysate gave the greatest production enhancement, subsequent studies proved that, in contrast to the CHO cell line, the hybridoma cell line performed best with the individual wheat hydrolysate. These data illustrate the value in evaluating both individual and blended hydrolysates to address cell line specific variation in nutritional requirements. With this information, a proper optimization study can be designed to achieve the production benefits of hydrolysate supplementation.

Figure 1: CHO Line #1 Hydrolysate Blending Study
Antibody Yield Determined by ELISA

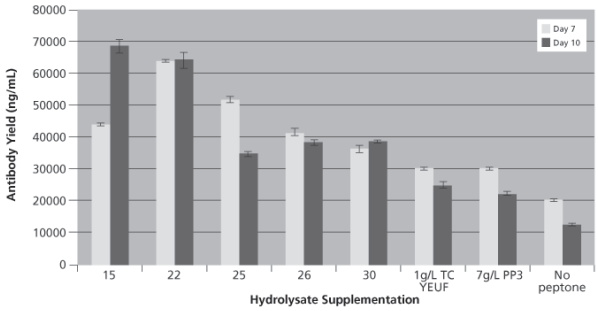
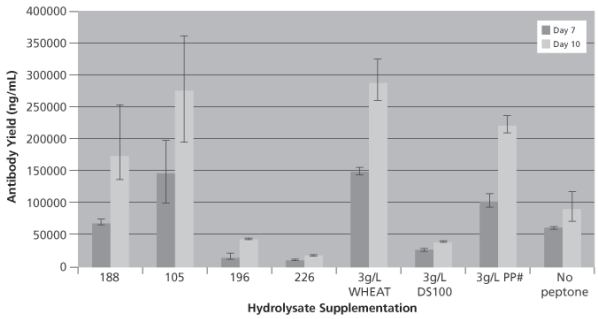


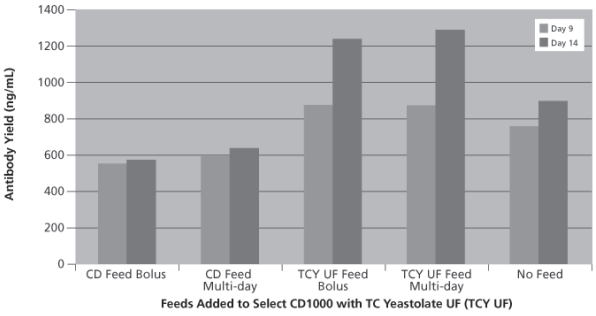
Figure 2: Hybridoma Hydrolysate Blending Study
Antibody Yield Determined by ELISA



Case Study #2 — Comparison of Hydrolysate Supplementation and Feeds to Chemically Defined Feeds

Using BD's Autonutrient™ Media Design Service (AMDS), an optimized chemically defined base medium was developed for CHO Line #2. The goal of this study was to identify an animal free hydrolysate supplementation and feed strategy for enhanced antibody production. The optimized base medium supplemented with TCY UF was used as the starting medium and fed with TCY UF or a chemically defined (CD) feed on various days or as a bolus. As shown in Figure 3, a bolus feed or multiple feeds of TCY UF gave significantly enhanced production compared to CD feeds or the control without a feed. This study illustrates the time savings of using a hydrolysate feed versus the labor intensive process of identifying CD feeds.

Figure 3: CHO Line #2 Hydrolysate Feed Study
Antibody Yield Determined by Protein A HPLC



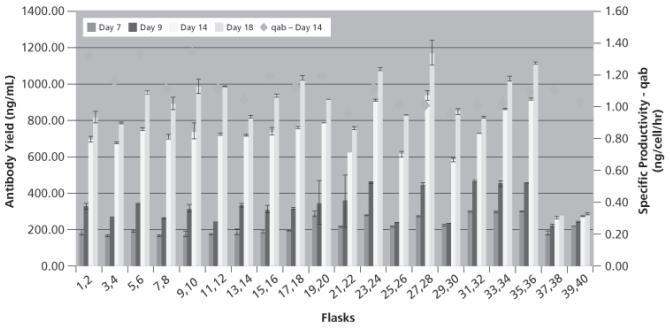
Case Study #3 — Hydrolysates Used Only as a Feed

The goal of this study was to identify an animal-free hydrolysate based feed strategy for enhanced antibody production from CHO Line #3 using an in-house CD medium. Cells were seeded at two different densities the CD medium and an individual hydrolysate was fed at varying concentrations as a bolus on day 5 and/or day 7 (Figure 4). As shown in Figure 5, all concentrations and feed strategies enhanced antibody production above controls with no feed. Flasks 27 and 28 resulted in the highest production level using 17 g/L of hydrolysate fed on day 5. This data illustrates the importance of evaluating a wide range of hydrolysate concentrations to ensure the proper concentration has been identified. The data also demonstrates the benefit of determining the appropriate addition time of hydrolysate in the process.

Figure 4: Flask Key for CHO Line #3 Hydrolysate Feed Study in Figure 5

Flasks	Seeding Density	Hydrolysate Feed Concentration	Feed Date
1,2	2.00E+05	9 g/L (4.5 g/L per timepoint)	5
3,4	2.00E+05	9 g/L	7
5,6	2.00E+05	13 g/L	5
7,8	2.00E+05	13 g/L	7
9,10	2.00E+05	17 g/L	5
11,12	2.00E+05	17 g/L	7
13,14	2.00E+05	9 g/L (4.5 g/L per timepoint)	5 & 7
15,16	2.00E+05	13 g/L (6.5 g/L per timepoint)	5 & 7
17,18	2.00E+05	17 g/L (8.5 g/L per timepoint)	5 & 7
19,20	4.00E+05	9 g/L	5
21,22	4.00E+05	9 g/L	7
23,24	4.00E+05	13 g/L	5
25,26	4.00E+05	13 g/L	7
27,28	4.00E+05	17 g/L	5
29,30	4.00E+05	17 g/L	7
31,32	4.00E+05	9 g/L (4.5 g/L per timepoint)	5 & 7
33,34	4.00E+05	13 g/L (6.5 g/L per timepoint)	5 & 7
35,36	4.00E+05	17 g/L (8.5 g/L per timepoint)	5 & 7
37,38	2.00E+05	No Feed	NA
39,40	4.00E+05	No Feed	NA

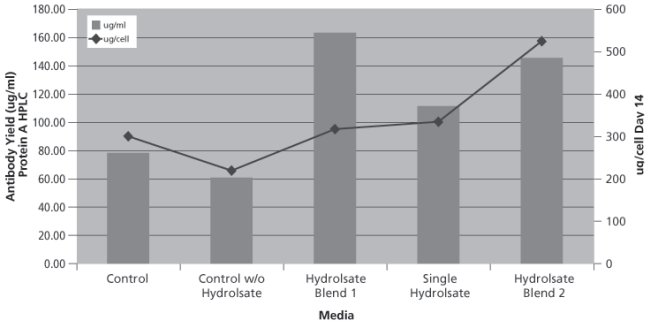
Figure 5: CHO Line #3 Hydrolysate Feed Study Antibody Yield Determined by Protein A HPLC



Case Study #4 — PerC6 Hydrolysate Screen

Using AMDS methods, a CD base medium was optimized for a PerC6 cell line. The goal of this study was to identify a hydrolysate supplementation for this medium to increase antibody production relative to the control medium. A panel of animal free hydrolysates were evaluated, either individually or as blends, to identify the optimal supplement for antibody production enhancement. Figure 6 shows that an individual hydrolysate (Single Hydrolysate), as well as two blends of hydrolysates (Hydrolysate Blend 1 and Hydrolysate Blend 2) enhanced antibody production compared to the control media. Although Hydrolysate Blend 1 produced the highest volumetric antibody yield, the associated specific productivity was equal to or less than the specific productivities for both the Single Hydrolysate and Hydrolysate Blend 2. These data support the need to evaluate both individual and blends of hydrolysates, as well as the importance of evaluating the potential effects the supplementation strategy has on both cell growth and productivity.

Figure 6: Peptone Feed Timing Study Antibody Yield Determined by Protein A HPLC

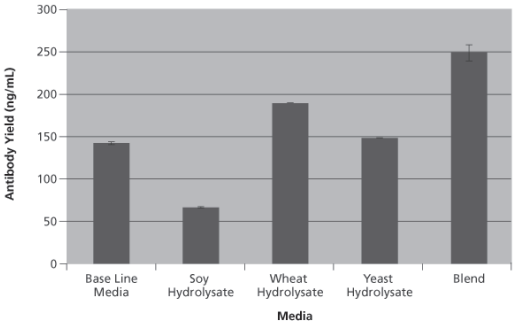


METHODS AND RESULTS CONTINUED

Case Study #5 – NS0 Hydrolysate Screen

Using AMDS methods, a CD base medium was developed for an NS0 cell line. The goal of this study was to identify hydrolysate supplementation for an in-house CD medium to increase antibody production. A panel of animal-free hydrolysates were evaluated in the CD medium, either individually or as blends, to identify the optimal supplement for antibody production enhancement. Figure 7 shows that, although the individual wheat or yeast hydrolysate gave slight increases in antibody production, a blend of multiple hydrolysates produced approximately a 1.75-fold increase in antibody production. Production benefits can be achieved when more than two hydrolysates are blended, so they should be considered as part of a thorough optimization process.

Figure 7: NSO Hydrolysate Study
Day 12 Antibody Yield Determined by Protein A HPLC



CONCLUSIONS

Hydrolysates are an effective, cost effective, and proven option for rapid media supplementation and process optimization. A successful optimization strategy includes hydrolysates screened individually and blended in an optimized medium. As demonstrated in these studies, hydrolysate blends can provide synergistic enhancement of protein production levels. Hydrolysate feed strategies are cell line and process dependent, so it is important to screen a wide variety of hydrolysates at varying concentrations across multiple feed strategies. As summarized in Figure 8, these case studies illustrate that supplementation with hydrolysates can greatly increase protein production across multiple cell lines in minimal time.

Figure 8: Case Study Summary

Cell Line	Hydrolysate Supplementation Strategy	Optimal Results	Improvement Over Control
CHO Line #1	Hydrolysate Blending	Blend 15 - Blend of Soy and Yeast Hydrolysates	2-fold increase in antibody production
Hybridoma Line	Hydrolysate Blending	Wheat Hydrolysate Alone	3-fold increase in antibody production
CHO Line #2	Hydrolysate Supplementation and Feed	Hydrolysate Addition on Days 0 and 3	1.4-fold increase in antibody production
CHO Line #3	Hydrolysate Supplementation and Feed	Hydrolysate Addition only on Day 5	7-fold increase in antibody production
PerC6 Line	Hydrolysate Blending	Hydrolysate Blend 1	2-fold increase in antibody production
NS0 Line	Hydrolysate Blending	Blend of All Three Hydrolysate	1.75-fold increase in antibody production