Platform Versus Specific Hydrolysate Optimization For Three CHO Cell Lines: A Feasibility Study

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Abstract

A common question about culture media optimization is whether a generic platform system can effectively substitute for cell line specific systems. It is generally accepted that for maximum performance a specific set of media should be developed for each cell line. This can be costly and time consuming; the use of a suitable platform system may be preferential. This case study was designed to evaluate the feasibility of using a platform blend of hydrolysates as a media supplement for three recombinant CHO cell lines rather than blends optimized for each line. The blends were composed of hydrolysates from three plant and microbial sources. Cell line specific blends were formulated using statistically designed mixtures experiments and a platform blend was determined by simultaneous optimization for all three cell lines. An acceptance criterion for the platform blend was set at 85% of performance compared to the specific blends for all three cell lines in regards to cell growth and production. Performance in the platform blend for each cell line was >90% compared to the specific blends, meeting the acceptance criteria. Evaluation of the platform blend with additional cell lines should be conducted for further proof of concept.

Methods

Sequence of Development

The study was divided into two phases.

First, an optimized hydrolysate blend was developed for each of three recombinant CHO cell lines (see Cell Culture). To allow for development of the platform blend, the same experimental design and conditions were used for all three cell lines.

Second, a platform hydrolysate blend was predicted using the response data from all three cell lines. Performance using this platform blend was compared with the individually optimized blends using all three cell lines. The criterion for feasibility of the platform blend was set at ≥85% performance of each cell specific blend.

Cell Culture

Three recombinant CHO cell lines were used in this study. The three cell lines were derived from the same parent cell line but produced different products. Cell culture was carried out in 125mL shaker flasks with a working volume of 30mL culture media on an orbital shaker platform set at 120 RPM in an incubator set at 37°C and 5% CO_2. The same proprietary animal-component-free and chemically-defined basal culture medium was used for all three cell lines. 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). 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 the cultures. Cultures were terminated once viability dropped below 70% and samples were taken for quantification of production using protein-G affinity chromatography.

Statistically Designed Mixtures Experiments (DoE)

Three non-animal derived hydrolysates from different sources were blended in varying proportions using a statistically designed mixtures experiment (DoE; Table 1). The total concentration for each mixture was 6g/L. Design Expert® version 7.1.3 was used for experimental design and analysis. The experiment was designed using a d-optimal mixtures design with a partial cubic model. The responses evaluated in this experiment were cell growth (cumulative cell density) and volumetric production (mg/L). A quadratic regression model was used to perform surface response analysis on the data. The overall goal of the optimization was to maximize cell growth and production.

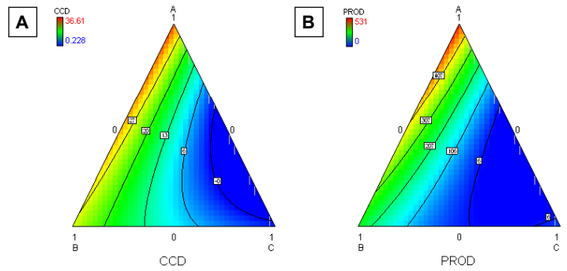
Table 1. Design Parameters of DoE Mixtures Experiments

Parameter	Value
Design	D-optimal Special Cubic model
Analysis	Mixture Quadratic model
Number of Design Points	24
Factors	Hydrolysates A, B, and C
Responses	Cumulative Cell Density (CCD), Volumetric Production

1. Prediction of Cell Line Specific Hydrolysate Blends.

The optimal hydrolysate blend for each cell line was predicted using surface response analysis for cell growth (CCD) and volumetric production (PROD) responses (Figures 1 – 3). The highest ranked optimal hydrolysate blend prediction is listed for each cell line with desirability. Desirability is a reflection of the suitability of a predicted optimal condition for multiple responses and is shown as a value between 0 and 1.

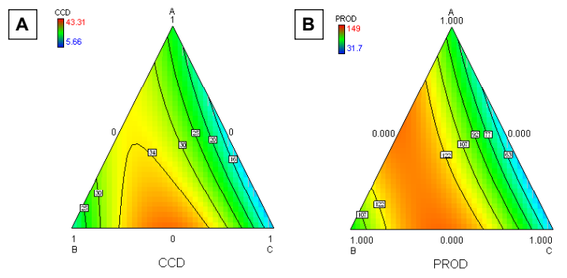
Figure 1. Cell Growth (A), Production (B), and Predicted Hydrolysate Blend (C) for Cell Line 1. Cell growth is shown as cumulative cell density (CCD).



C

Cell Line 1	Hydrolysate			Desirability
	A	B	C	
Prediction	100%	0	0	0.94

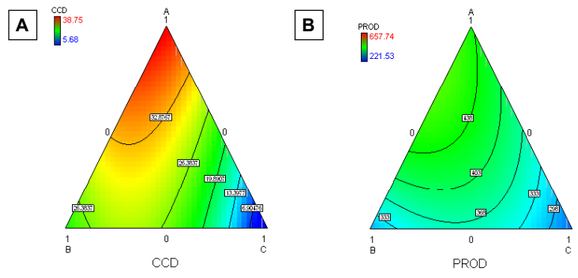
Figure 2. Cell Growth (A), Production (B), and Predicted Hydrolysate Blend (C) for Cell Line 2. Cell growth is shown as cumulative cell density (CCD).



C

Cell Line 2	Hydrolysate			Desirability
	A	B	C	
Prediction	0%	50%	50%	0.89

Figure 3. Cell Growth (A), Production (B), and Predicted Hydrolysate Blend (C) for Cell Line 3. Cell growth is shown as cumulative cell density (CCD).



C

Cell Line 3	Hydrolysate			Desirability
	A	B	C	
Prediction	90%	10%	0	0.69

2. Prediction of a Platform Hydrolysate Blend

To design the platform hydrolysate blend, surface response analysis was conducted to predict the best blend for all three experimental cell lines. The most desirable prediction for the platform hydrolysate blend was a blend of 66% A and 33% B (Figure 4). The top two ranked predictions for each cell specific and platform blend were used for experimental verification and comparison (Table 2).

Figure 4. Prediction of the Optimal Platform Hydrolysate Blend. A surface response desirability plot is shown. The first ranked blend prediction was 66% A and 33% B (red diamond); the second was 50% A and 50% B (blue diamond).

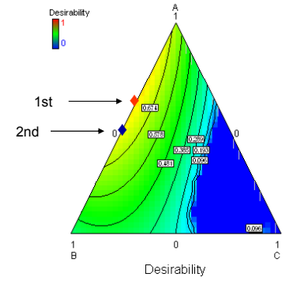


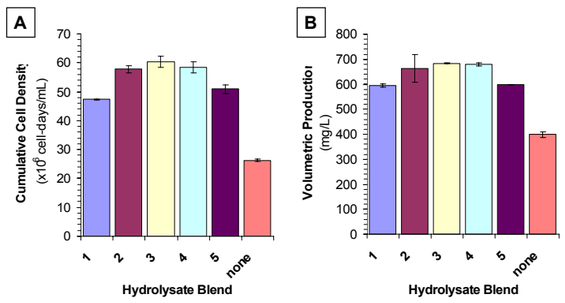
Table 2. Summary of Hydrolysate Blend Predictions for the Platform and Individual Cell Lines. For each prediction the ranking is given with the desirability in parentheses. The grayed out boxes represent undesirable conditions.

	Hydrolysate Blend Composition			Predictions for Platform and Cell Specific Blends			
Blend	A	B	C	Platform	1	2	3
1	90%	10%					1 (0.69)
2	50%	50%		2 (0.76)	2 (0.78)	2 (0.83)	2 (0.64)
3	66%	33%		1 (0.78)			
4	100%				1 (0.94)		
5		50%	50%			1 (0.89)	

3. Blend Performance and Platform Selection

Cell growth and production in the five predicted hydrolysate blends (Table 2) and a control medium (without hydrolysates) were evaluated (Figures 5-7). Given the performance criterion, Blend 2 was chosen as the Platform (Table 3). Cell growth and Production in this Platform were ≥93% compared to the top performing blend in all cell lines.

Figure 5. Cumulative Cell Density (A) and Volumetric Production (B) of Cell Line 1 in the Hydrolysate Blends. Data shown is the average of three replicate flasks.



3. Blend Performance and Platform Selection (cont.)

Figure 6. Cumulative Cell Density (A) and Volumetric Production (B) of Cell Line 2 in the Hydrolysate Blends. Data shown is the average of three replicate flasks.

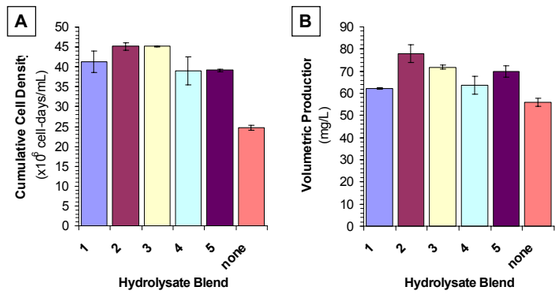


Figure 7. Cumulative Cell Density (A) and Volumetric Production (B) of Cell Line 3 in the Hydrolysate Blends. Data shown is the average of three replicate flasks.

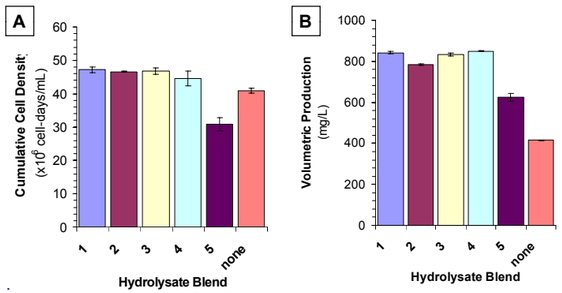


Table 3. Performance Comparison of the Platform and Top Performing Blend for each Cell Line. Performance of all cell lines in the Platform Blend (Blend 2) met the acceptance criterion of 85% of the top performing blend.

	Growth		Production	
Cell Line	CCD ($x10^6$cell-days/mL)	Percent of Top Performer	Production (mg/L)	Percent of Top Performer
1	58	96%	681	97%
2	45	100%	78	100%
3	47	99%	784	93%

Summary & Conclusions

1. **The feasibility of using a platform hydrolysate blend rather than cell line specific blends was evaluated using three recombinant cell lines.**
2. **Cell line specific blends and a platform blend were developed using statistically designed mixtures experiments.**
3. **Performance in the platform blend for each cell line was ≥93% compared to the specific blends, exceeding the acceptance criteria that was set at ≥85%.**
4. **Evaluation of the platform with additional cell lines is necessary to provide proof of concept.**

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