A Novel Approach to Rapid Feed Strategy Optimization

Evan R. Horowitz, Mark C. Arjona, Jenny Y. Bang, and Scott D. Storms
Irvine Scientific, Santa Ana, CA, USA

Abstract

Feed strategy can have a significant impact on overall culture performance of recombinant cell lines and its development is integral in fed-batch process development. Designing an effective feed strategy can be complex and time consuming as it involves balancing multiple factors, including the feed media, feed schedule, and feed volume. Using a traditional one-factor-at-a time methodology, the design of an optimized feed strategy for a straightforward single feed process can require four to ten experiments. A simplified, rapid method was designed to expedite feed strategy development in only four experiments, significantly reducing the time required by as much as fifty percent. The method includes two initial characterization experiments to devise an initial feed strategy in terms of feeding start day and total feed volume. The third and key experiment utilizes a statistical approach (design of experiment; DoE) and provides a predicted optimal feed strategy. The fourth experiment is conducted to verify the resulting predictions. The factors optimized for were feeding duration (days), daily feed volume (v/d), and feed starting day. To demonstrate feasibility of this method, it was employed to develop feed strategies for three recombinant CHO cell lines. In order to compare the effectiveness of the new method, both traditional and new approaches were used for each cell line. For all three cell lines tested, the platform method led to optimized feed strategies that were similar between the traditional and new methods. It is suggested that this method can be implemented as a platform for rapidly determining an optimized feed strategy for a variety of cell lines utilizing fed-batch culture processes. In addition to the reduction in development timelines, this method provides insight into interactions between each factor that can lead to further improvement in optimization compared to the traditional method.

Methods

Experimental Approach

Designing an optimal feed strategy can be a laborious process when using traditional one-factor-at-a-time (OFAT) methodologies. A standard feed strategy development project can be conducted with eight experiments (Table 1). In order to conserve time and resources a methodology utilizing Design of Experiment technology (DoE) was developed to optimize feed strategy in only four experiments (Table 2).

Table 1. Typical Feed Strategy Development Timeline Using OFAT Methodology.

Experiment	1st Month	2nd Month	3rd Month	4th Month	5th Month	6th Month
Characterization	20 Days					
Basic Feed Strategy	21 Days					
Feed Concentration	24 Days					
Daily Feed Volume	24 Days					
Feed Timing	22 Days					
Feed Concentration II	21 Days					
Daily Feed Volume II	21 Days					
Feed Strategy Verification	24 Days					

Table 2. Novel Feed Strategy Development Timeline Using DoE Methodology.

Experiment	1st Month	2nd Month	3rd Month
Characterization	20 Days		
Feed Concentration	24 Days		
Feed Strategy DoE	20 Days		
Feed Strategy Verification	24 Days		

Feasibility Study

Three recombinant CHO cell lines producing antibody or non-antibody proteins were selected for the feasibility study. All three had undergone feed strategy optimization using the OFAT methodology. For each cell line, the new DoE feed strategy optimization strategy was employed. Feed Concentration, Feed Strategy DoE, and Feed Strategy Verification experiments were conducted and compared to the results obtained using the OFAT methodology. The original data for the Characterization experiment was common to both methods and was used for the Feasibility Study. All experiments were performed in shaker flasks (suspension culture) using media and culture parameters optimized for each cell line.

rCHO Cell Line 1

The first recombinant cell line expresses a non-antibody protein and had a compressed project timeline. Fed-batch cultures conducted using the OFAT optimized feed strategy resulted in a modest improvement in cell growth (Figures 1A & 1B) and more than a doubling of production (Figure 1B) compared to batch cultures. The new DoE feed strategy optimization method was performed (Figures 2 – 3) and different values compared to the OFAT method were obtained (Figure 3C). The verification experiment resulted in superior performance with the new (DoE) method for both cell growth and production (Figures 4A & 4B).

Figure 1. Growth and Production in Fed-Batch Cultures Optimized Using OFAT Methodology Compared to Batch Cultures. Cell growth curves (A) and culture performance relative to batch of Cumulative Cell Density and Volumetric Production (B). Data shown is the average of 3 replicates per condition.

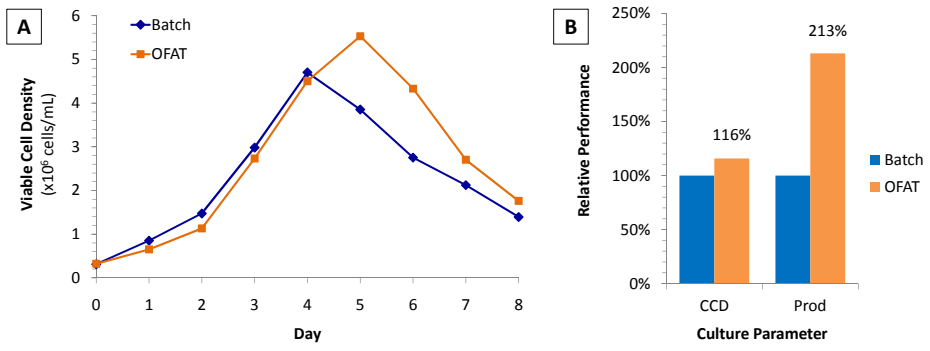


Figure 2. Feed Volume Experiment – Cell Growth and Production. Data is shown relative to batch cultures for Cumulative Cell Density (CCD) and volumetric production (Prod) and is the average of 3 replicates per condition ± standard deviation .

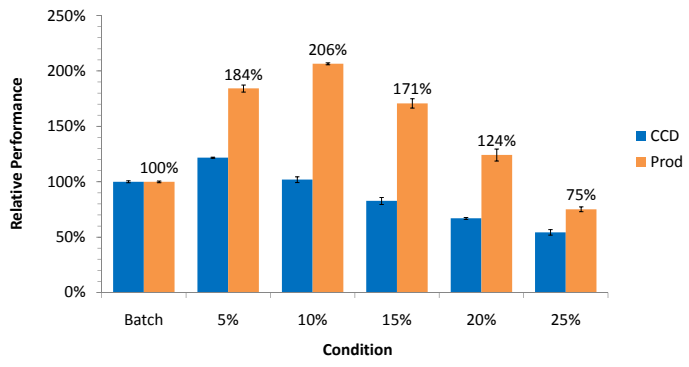


Figure 3. DoE Feed Parameter Experiment – ANOVA (A), Desirability (B), and Predictions (C).

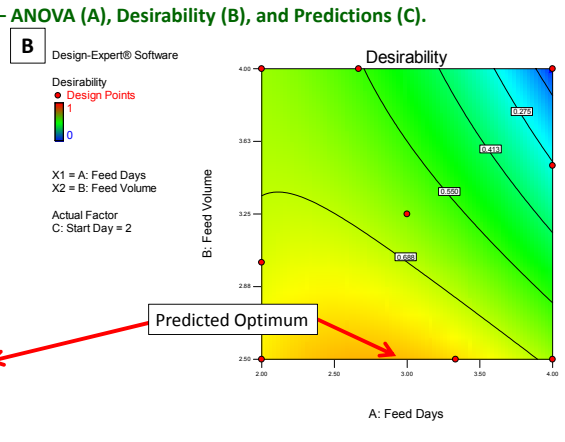
A

Cell Line 1 ANOVA Summary		
	Effect	
Parameter	CCD	Vol Prod
Model	***	***
Feed Days	***	
Feed Volume	***	
Start Day	***	***
Lack of Fit	*	

Legend: *** p<0.001, ** p<0.01, * p<0.05

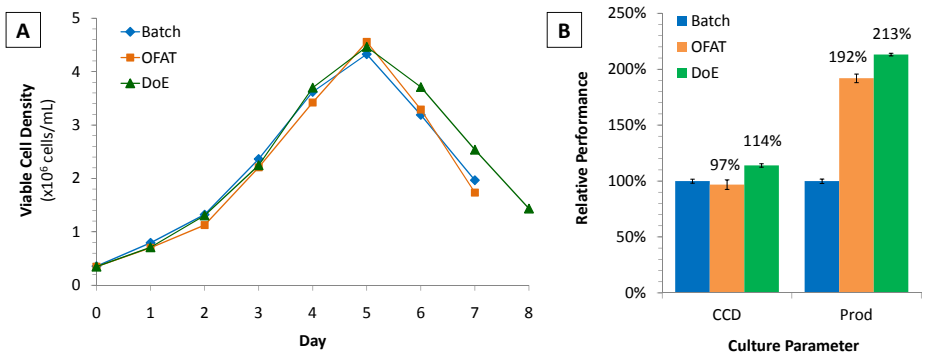
C

Predictions – Feed Strategy by Method		
Parameter	OFAT	DoE
Feed Days	5	3
Feed Volume	2% wv	2.5% wv
Start Day	2	2



rCHO Cell Line 1 (continued)

Figure 4. Verification Experiment – Comparison of OFAT and DoE Methodologies. Cell growth curves (A) and culture performance relative to batch of Cumulative Cell Density and Volumetric Production (B). Data shown is the average of 3 replicates per condition and error bars (B) are ± standard deviation.



rCHO Cell Line 2

The second recombinant CHO cell line expresses an antibody protein and had a standard project timeline. Using the DoE method, Characterization and Feed Concentration experiments (data not shown) were conducted to determine the feed start day range and total feed concentration. The Feed Strategy DoE led to two predictions including one that was similar to the results of the OFAT method (Figure 5). The Verification experiment resulted in equivalent performance with either method (Figure 6).

Figure 5. DoE Feed Parameter Experiment – ANOVA (A), Desirability (B), and Predictions (C).

A

Cell Line 2 ANOVA Summary		
	Effect	
Parameter	CCD	Vol Prod
Model	***	***
Feed Days	***	***
Feed Volume	***	***
Start Day		*
Lack of Fit		

Legend: *** p<0.001, ** p<0.01, * p<0.05

C

Predictions – Feed Strategy by Method		
Parameter	OFAT / DoE 1	DoE 2
Feed Days	7	5
Feed Volume	3% wv	4% wv
Start Day	3	2

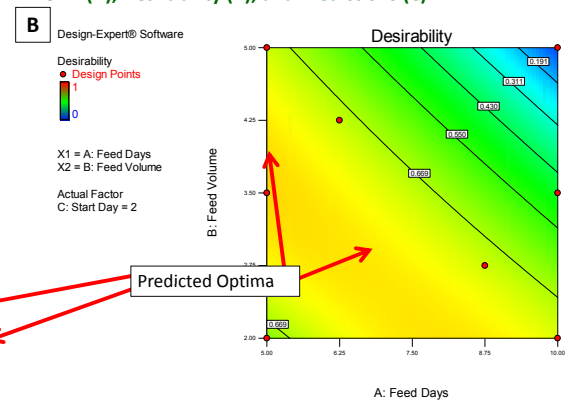
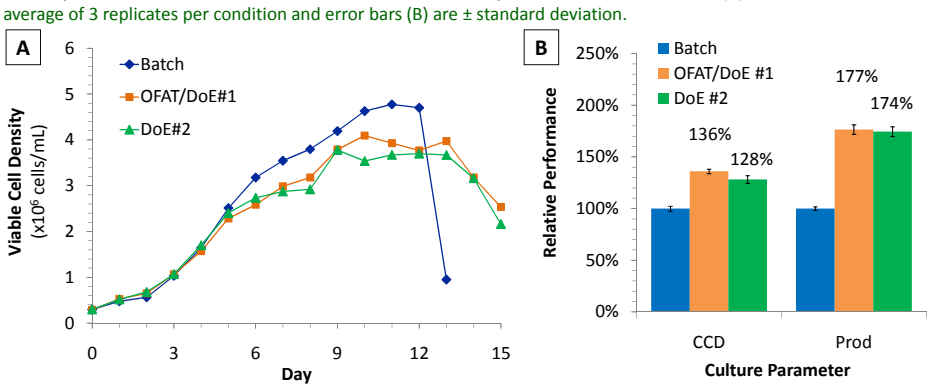


Figure 6. Verification Experiment – Comparison of OFAT and DoE Methodologies. Cell growth curves (A) and culture performance relative to batch of Cumulative Cell Density and Volumetric Production (B). Data shown is the average of 3 replicates per condition and error bars (B) are ± standard deviation.



rCHO Cell Line 3

The third recombinant CHO cell line expresses an antibody protein and had a compressed project timeline. Using the DoE method, Characterization and Feed Concentration experiments (data not shown) were conducted to determine the feed start day range and total feed concentration. The Feed Strategy DoE led to a prediction significantly different than the OFAT method (Figure 7). The Verification experiment resulted in increased performance with the DoE method for both cell growth and production (Figure 8).

Figure 7. DoE Feed Parameter Experiment – ANOVA (A), Desirability (B), and Predictions (C).

A

Cell Line 3 ANOVA Summary		
	Effect	
Parameter	CCD	Vol Prod
Model	***	***
Feed Days		***
Feed Volume	**	***
Start Day	***	
Lack of Fit	**	*

Legend: *** p<0.001, ** p<0.01, * p<0.05

C

Predictions – Feed Strategy by Method		
Parameter	OFAT	DoE
Feed Days	6	6
Feed Volume	2% wv	4.7% wv
Start Day	2	2

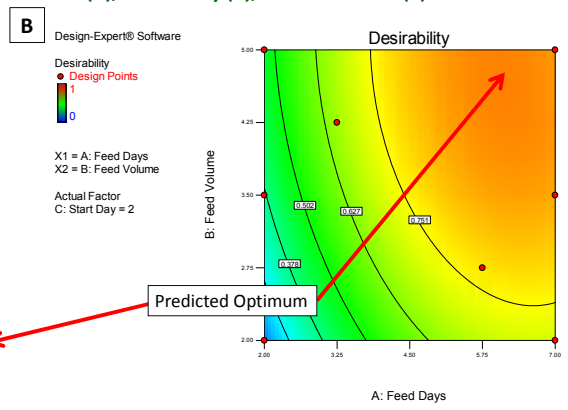
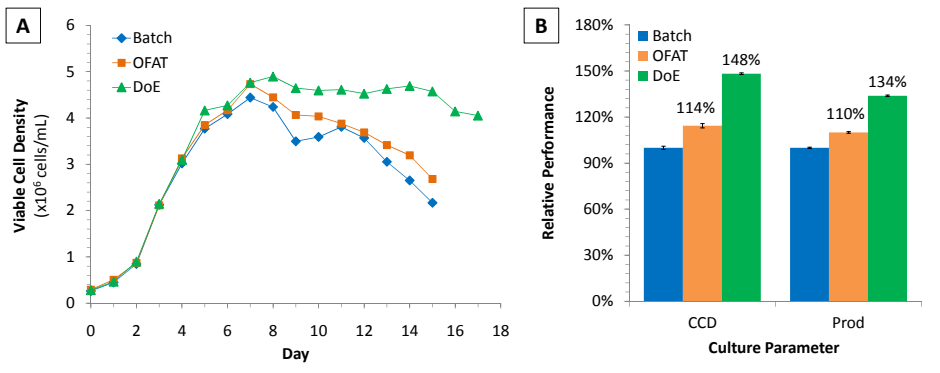


Figure 8. Verification Experiment – Comparison of OFAT and DoE Methodologies. Cell growth curves (A) and culture performance relative to batch of Cumulative Cell Density and Volumetric Production (B). Data shown is the average of 3 replicates per condition and error bars (B) are ± standard deviation.



Summary & Conclusions

1. **An optimized Feed Strategy is a critical aspect of an effective culture process and can be developed using OFAT methodology in eight experiments.**
2. **A novel DoE Feed Strategy approach was designed reducing the experiments to four.**
3. **The DoE method led to equivalent or better culture performance for all three rCHO cell lines.**
4. **Interestingly, each cell line responded differently in terms of cell growth, production, and specific productivity to Feed Strategy optimization.**

Presented at the 21st ESACT meeting, "Cellular Solutions for Clinical Challenges". Dublin Ireland, June 2009.