CHOptimizer®: The Media Builder

A Unique Rapid Approach for Media and Feed Optimization in ambr®15 Bioreactors

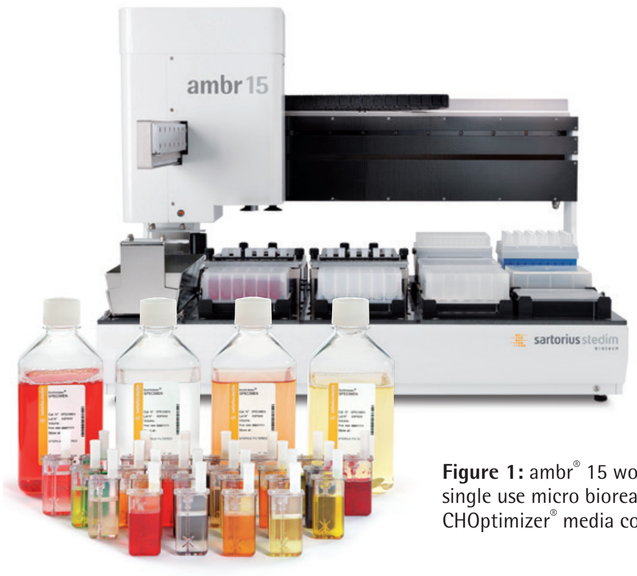

Figure 1: ambr® 15 workstation with single use micro bioreactors and CHOptimizer® media components

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Abstract
CHOptimizer® includes media application specialist guidance along with a portable ambr® 15 workstation that is brought onsite to optimize a CHO fed-batch process. After using CHOptimizer®, a 17 day fed-batch process was developed. From simple fed-batch (glucose only) to fed-batch, in less than 6 months an IgG1 producing cell line peak VCD was improved 2.5-fold from 4.5 × 106 cells/mL to 11 × 106 cells/mL and titer was improved 4-fold from 0.5 g/L to 1.9 g/L.

Introduction
Biopharmaceutical companies today are challenged to develop high producing cell lines as quickly as possible. Commercially available media may fall short of performance expectations in order to meet targets. Alternatively, fully customized media and feed development requires significant funding, time, and in house expertise in media development. To address these challenges CHOptimizer® offers dedicated media application specialists support, who guide customers through a series of experiments designed to optimize a fed-batch process using prequalified blends of well-characterized, Chemically Defined (CD), and Animal Origin Free (AOF) media. CHOptimizer® combines the performance of Lonza cell culture media with the power of the ambr® 15 workstation which facilitate high throughput Design of Experiments (DoE) analysis.

Phase I: Materials and Methods

Cell Line and General Parameters
CHOptimizer® was used to optimize a process for a CHO DG44 cell line producing an IgG1 antibody. Each phase involved at least two adaption passages in vented Erlenmeyer shake flasks and the media conditions for that experiment. The cells were then inoculated into the ambr® 15 micro bioreactors. An ambr® 15, 24 bioreactor culture station was used for all three phases.

Parameter	Description | Set Point
Expansion Vessel	Erlenmeyer shake flasks
Seed Density	0.2×10^6 (viable cells/mL)
Vessel Type	Sparge-less ambr® 15
Agitation (RPM)	1600

Measurements
Cell count and viability was measured using a Cedex HiRes automated cell counter. Metabolites as well as IgG titers were measured using a Cedex Bio. Data analysis and modeling was performed with MODDE 10 by Umetrics.

Phase I: Mixture DoE to Determine Basal Media Formulation
CHOptimizer® media consists of four CD and AOF base media with varied nutrient levels. The formulations are designed so that a 24 condition mixture DoE covers a wide range of concentrations. Figure 2 visualizes the D-optimal mixture design for the four base media that was used in Phase I (three center points not shown). Cultures were run in a simple fed-batch mode in ambr® 15 bioreactors where only glucose was fed to maintain concentrations above 2 g/L. Peak Viable Cell Density (VCD), Doubling Time (DT), and final IgG titer responses were analyzed using MODDE. Based on this analysis, three optimized media formulations were selected for use in Phase II.

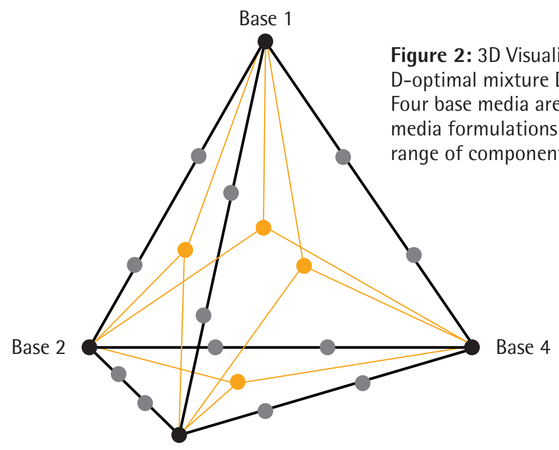

Figure 2: 3D Visualization of the D-optimal mixture DoE used in Phase I. Four base media are mixed to create media formulations that cover a wide range of component concentrations.

Phase I: Results

Phase I: Mixture DoE to Determine Basal Media Formulation
The simple fed-batch cultures were run for 10 to 14 days and each reactor was terminated when viability dropped below 50%. Table 1 summarizes the range of responses observed that were used for analysis. Figure 3 is the Observed vs. Predicted values for the model generated in MODDE. Titer and Peak VCD had strong model fit (R2) and predictability (Q2) while DT had a satisfactory model fit and significant but not strong Q2. MODDE was used to predict three optimal media formulations. The contour plots in Figure 4 show the predicted responses for three chosen mixtures of media. Table 2 presents the three media formulation chosen to move forward into Phase II and which parameters the media was optimized for. Media A was optimized for titer production, Media B was optimized for doubling time, and Media C was optimized for all three responses weighted equally.

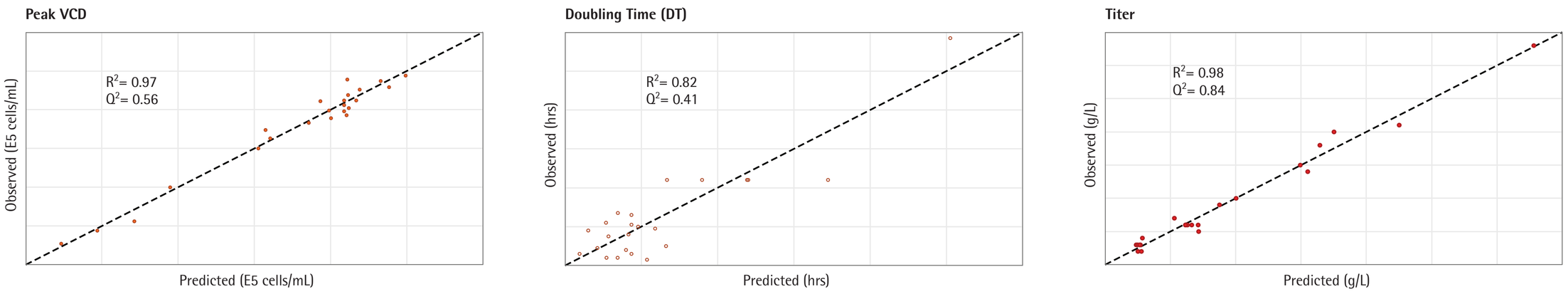

Figure 3: Plot of observed vs predicted values of three responses: Peak VCD, DT and titer

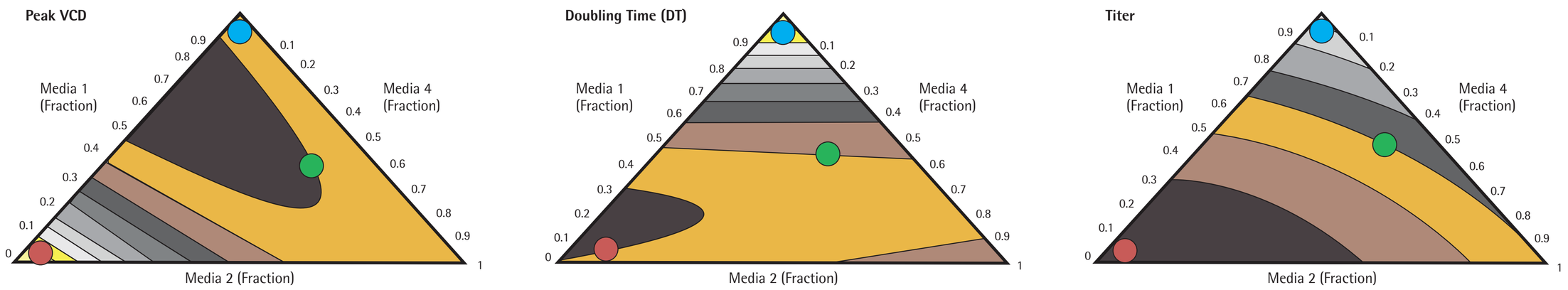

Figure 4: Contour plots for the predicted range of three different responses: Peak VCD, DT, and Titer. Media 3 = 0%. The three selected media were optimized for (A) production only (titer), (B) growth rate (DT), and (C) production, growth rate, and peak VCD weighted equally.

Response	Range	Average
Peak Viable Cell Density (X106 vc/mL)	1.8 to 4.0	3.3
Titer (g/L)	0.27 to 0.58	0.34
Doubling Time (hrs)	22 to 34	25

Table 1: Summary of Results of three responses from Phase I

		Predicted		
Symbol	Media Optimized for	Titer (g/L)	Peak VCD ($\times 10^6$ cells/mL)	DT (hrs)
●	Titer	0.6	3.6	32
●	Doubling Time	0.3	2.4	22.4
●	Titer, Peak VCD, and Doubling time	0.4	3.7	24.9

Table 2: Three Optimized Media Formulations and Predicted Outcomes

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Phase II: Materials and Methods

Phase II: Spent Media Analysis and Process Development
Three optimized media formulations were selected based on the DoE analysis of Phase I. As shown in Table 1, Phase II is a full factorial DoE to evaluate the addition of glutamine, and the effect of pH set points. The conditions were run in simple fed-batch mode (glucose only) in ambr® 15 bioreactors. Spent media analysis was performed and used to develop feeds based on component consumption rates. Spent media analysis was reviewed with support of the media application specialist and the Lonza development team to design the feeds.

Factor	Levels		
Media	A	B	C
pH range	6.6 to 6.8	6.9 to 7.1	
Glutamine supplemented (1 mM)	Yes	No	
Replicates	2 of each condition		

Table 1: Phase II DoE: Factors Tested in Simple Fed-batch (glucose only) – Spent media analysis performed for feed development.

Phase II: Results

Phase II: Spent Media Analysis and Process Development
Figure 1 is a graphical representation of the cell growth and titer for Phase II comparing the averaged conditions with the same pH set point. Additional glutamine supplementation showed no difference in cell growth or titer, as seen by the narrow standard deviation error bars in the graphs. The higher pH 7.15 condition resulted in improved growth during early culture days. Final titers were similar regardless of pH set point or glutamine supplementation. Media B had 40% lower peak VCD and 80% lower titers than A and C. Therefore Media A and Media C were selected for feed development in Phase III. Spent media data was used to develop feeds for the selected optimized media. An overview of the amino acid consumption for the top two optimized media is shown in Table 2.

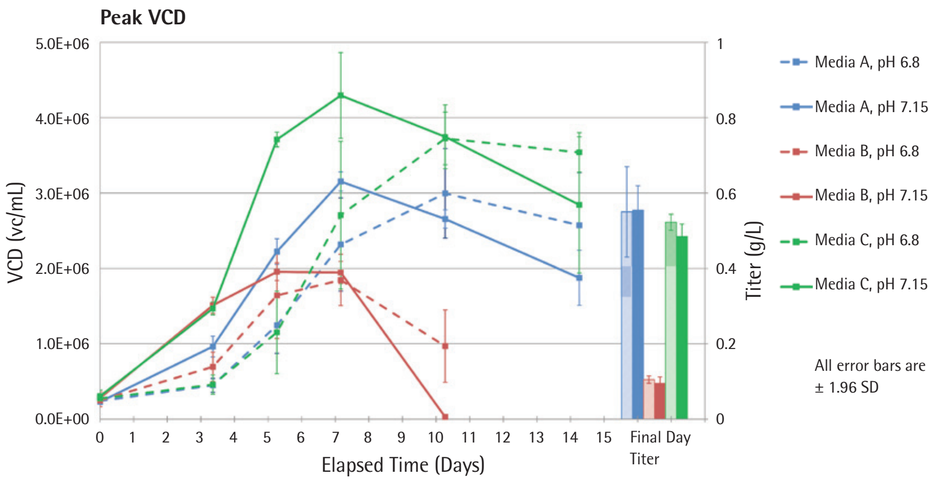

Figure 1: Phase II Cell Growth (VCD) and Final Day Titer. Averaged pH conditions graphed (N=4). Higher pH control resulted in better growth, but no improved production.

Colors	>100%	75% – 100%	50 – 75%	25 – 50%	< 25%
Media	Media A			Media C	
Day	3	5	7	14	3	5	7	17
L-Alanine								
L-Arginine								
Asparagine								
L-Aspartic acid								
Cystine								
L-Glutamic acid								
L-Glutamine								
L-Glycine								
L-Histidine								
L-Isoleucine								
L-Leucine								
L-Lysine								
L-Methionine								
L-Phenylalanine								
L-Proline								
L-Serine								
L-Threonine								
L-Tryptophan								
L-Tyrosine								
L-Valine								

Table 6: Phase II amino acid consumption relative to the initial concentrations for the two media that were selected for Phase III

Phase III: Materials and Methods

Phase III: Feed Strategy Development
Two media were selected, a pH set point of 7.15 was chosen, and glutamine was added based on the results of Phase II. Two feeds were developed based on the spent media analysis and consumption rates of the top two optimized media formulations. In Phase III, feed volume and timing were evaluated in a half fractional factorial DoE depicted in Table 2. The total feed volume was distributed evenly over 8 days (Example: 40% feed, daily feeds, starting day 3: results in 5% fed on days 3 through 10).

Parameter	Low	Center Points (4)				High
Media	A	A		C		C
Feed Formula	1	1	2	1	2	2
Feed Strategy	Every other day	Every other day				Daily
Feed Volume (% of initial bioreactor volume)	40	60				80
Feed Start Day	3	3				4

Table 2: Phase III DoE: Fed-Batch DoE for Feed Strategy Development – Feed quantity and timing of two feed formulations were evaluated for the top two optimized media.

Phase III: Results

Phase III: Feed Strategy Development
Peak VCD, Titer, and qP were used in MODDE to model and optimize the feed scheme. Media and feed volume had the most significant effects on titer and cell growth. The prediction plots in Figure 2 show the significant factors based on statistical analysis. Feed formulation and feeding daily vs. every other day were not significant and are not shown. The final process was optimized for maximum protein production.

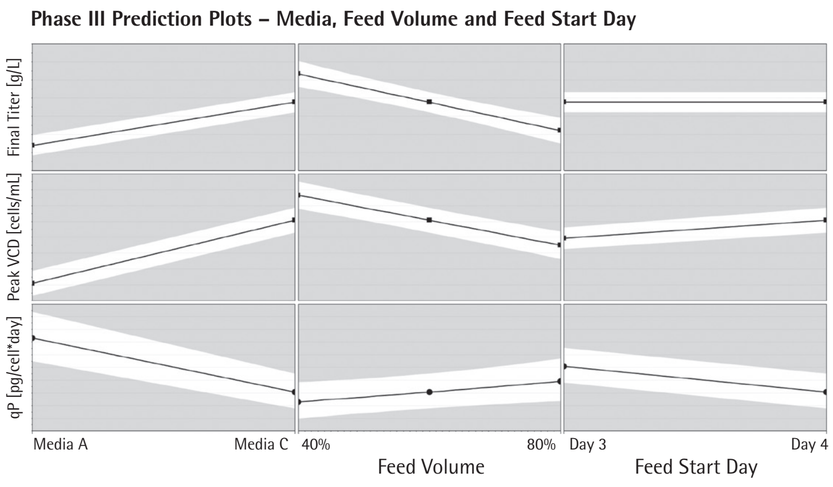

Figure 2: Prediction Plots: A horizontal line indicates that a factor did not have a significant effect on the response. Media formulation and feed volume had the greatest effects. Feed formulation and daily vs. every other day feeding were not significant.

Parameter	Optimal Setpoint
Media	C
Feed Formula	2
Feed Strategy	Every other day
Feed Volume (% of initial bioreactor volume)	40
Feed Start Day	3

Predicted Response	Model Prediction
Peak VCD ($\times 10^6$ cells/mL)	10
Titer (g/L)	2.1
qP (pg/cell*day)	20

Table 3: Summary of the optimized process parameters and predicted outcomes based on MODDE analysis.

VCD and titer for all three phases of CHOptimizer® are graphed in Figure 3. The data shown for Phase I is the closest mixture that was tested to the actual optimized mixture used in Phase II and III. The optimized fed-batch compared to the simple fed batch cultures showed about a 2.5-fold increase in peak VCD, and a 4-fold increase in titer. The selected conditions and predicted results based on the model are shown in Table 3. These predictions will be confirmed at bench top bioreactor scale in the future.

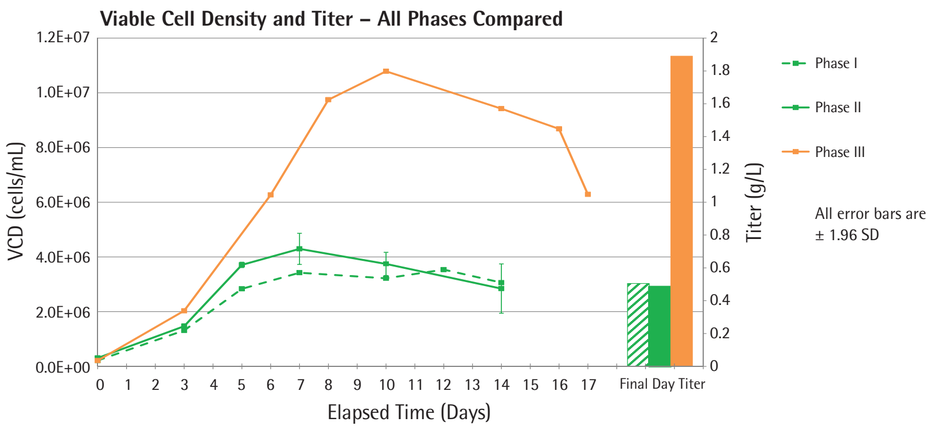

Figure 3: Three phases of media and feed optimization are shown. Green is Phase I and Phase II in simple fed-batch mode (glucose only). Orange is the final optimized fed batch process with 2.5-fold increase in VCD (11×10^6 cells/mL) and 4-fold increase in titer (1.9 g/L).

Conclusion

- Improvement from Phase I simple-fed batch cultures to Phase III fed-batch process was 2.5-fold increase in peak VCD and 4-fold increase in IgG titer
- Simple fed-batch controls demonstrated reproducibility of the process from Phase I to Phase III
- CHOptimizer® including the ambr® 15 and MODDE package effectively optimized media and feeds for a CHO fed-batch process in less than the 6 month projected timeline
- The experiments were guided by a field based Applications Specialist that supported the design and execution of the optimization through the full lifecycle of the project