SARTORIUS

Application Note

February, 2025

Keywords or phrases:
CHO media, fed-batch culture, protein based therapy, Ambr® 15, MODDE®, process optimization, design of experiments

CHO Cell Culture Optimization: A Design of Experiments Approach in the Ambr® 15

Miroslav Matev, Shiori Akiyama, Toru Tsuji, Satoshi Igarashi*
*Sartorius Stedim Japan K.K., 4F Daiwa Shinagawa North Bldg., 1-8-11, Kita-Shinagawa, Shinagawa-ku, Tokyo, 140-0001, Japan

Correspondence
Email: satoshi.igarashi@sartorius.com

Abstract

Culture media and feeding strategies significantly impact the productivity of monoclonal antibody production in CHO cell cultivations. Selecting the appropriate basal medium and finding the optimal process parameters is crucial for establishing a highly productive and robust production process. However, process optimization is often challenging in shake flasks due to limited labor resources and a lack of pH and DO control capability. Furthermore, poor scalability between shake flasks and large-scale bioreactors can also yield inconsistent results. The combination of a high throughput automated bioreactor system Ambr® 15 and a design of experiments (DOE) approach can accelerate this time-consuming and complex process.

In this application note, we present a case study of CHO cell culture process optimization using media, Ambr® 15, and the DOE software MODDE® from Sartorius. The initial media screening involved evaluating the performance of five different basal media and three feed media in shake flasks. The best-performing medium, called 4Cell® CHO Medium TCX6D, was selected and used in subsequent DOE experiments in the Ambr® 15, where optimal process parameters (e.g., pH, seeding density, and feeding strategy) were determined.

The best-performing media, in combination with the optimal process parameters and feeding strategy, resulted in a 1.7-fold increase in titer and final cell viability improvement compared to shake flask cultures. Using a systematic approach such as DOE and a high throughput automated bioreactor system similar to large-scale bioreactors greatly accelerated the optimization process, improved data quality, and reduced manual labor.

For further information, visit
sartorius.com/cho-media

Introduction

CHO cell lines are widely used to produce protein-based therapeutics such as monoclonal antibodies, enzymes, and hormones. Although the CHO-based protein production system was established over 30 years ago,[1] improving product titers remains a key area of interest. Cell culture media and process parameters significantly influence cell growth and productivity.[2] Therefore, identifying the optimal media and process parameters at an early stage is crucial for the efficient development of a highly productive and robust manufacturing process.

Optimal culture conditions are essential to enhance media performance. Numerous parameters contribute to productivity, e.g., pH, dissolved oxygen (DO), stirring speed, temperature, and feeding strategy.[3] Moreover, interaction effects among parameters are frequently observed; examining one factor at a time cannot provide sufficient insights to determine the optimal set points. A Design of Experiments (DOE) approach is a powerful tool that enables the simultaneous assessment of several factors and their interactions.

Sartorius' chemically defined CHO media portfolio includes seven basal media and six companion feeds suitable for the most common CHO cell lines, e.g., CHO-DG44, CHO-S, CHO-K1, and CHO-GS, in batch, fed-batch, and perfusion cultures.

The Ambr® 15 automated bioreactor system is an ideal tool for early process development, including media and feed optimization, as it supports 24 or 48 parallel cultivations at a 10–15 mL scale. The microbioreactor vessel mimics the characteristics of a large-scale bioreactor as each microbioreactor is equipped with pH and DO sensors, a central shaft blade impeller, and a sparge tube for gassing. Furthermore, feeding and sampling are executed by the automated liquid handler, which substantially reduces manual labor and improves consistency. In addition, the integration of DOE software (MODDE®) supports seamless experiment planning and execution.

We partnered with our client to conduct a study aimed at achieving an IgG1 titer of 5 g/L or higher using small-scale bioreactors. We first conducted media screening experiments in shake flasks to determine the best-performing media and subsequently carried out two consecutive process optimization runs using Ambr® 15 and MODDE®. The optimized process performance exceeded the goal of 5 g/L.

Materials

- CHO cell culture media and feeds are shown in Table 1
- GlutaMAX™ Supplement (ThermoFisher)
- Antifoam C (Sigma-Aldrich)
- 1 M sodium carbonate solution
- 450 g/L glucose solution
- Trypan blue
- Countess™ 3 Automated Cell Counter (ThermoFisher)
- Glucose analyzer
- Titer measurement – ELISA
- 125 mL baffled shake flasks (Corning)
- Ambr® 15 Cell Culture System, 24-way (Part#: 001-8B20)
- Ambr® 15 Cell Culture microbioreactors with sparger (Part#: 001-7B01)
- MODDE®

Table 1: *Media and Feeds Used in This Study*

Kit Name	Kit Part Number	Content	Category	Product Part Number
4Cell® CHO Media Kit	CFP3FF0201	4Cell® CHO Medium TCX6D	Basal medium	1070-0001
		4Cell® CHOlean Medium	Basal medium	1140-0001
		4Cell® CHO Medium TC-42	Basal medium	511-0001
		4Cell® Basic Feed	Feed	1092-0001
4Cell® CHO-GS Media Kit	CFP3FF0200	4Cell® CHO-GS TCX10D Medium	Basal medium	1150-0001
		4Cell® CHO Feed TCX7D	Feed	1080-0001
4Cell® XtraCHO Media Kit	CFP3FA0200	4Cell® XtraCHO SAM	Basal medium	CFP3FA1201
		4Cell® XtraCHO PM	Basal medium	CFP3FA2202
		4Cell® XtraCHO FMA	Feed	CFP3FA3203
		4Cell® XtraCHO FMB	Feed	CFP3FA4204
4Cell® SmartCHO Media Kit	CFP3FB0200	4Cell® SmartCHO SAM	Basal medium	CFP3FB1106
		4Cell® SmartCHO PM	Basal medium	CFP3FB2107
		4Cell® SmartCHO FMA	Feed	CFP3FB3108
		4Cell® SmartCHO FMB	Feed	CFP3FB4109

Note. 4Cell® SmartCHO is the newest version of 4Cell® XtraCHO. SAM = Stock and Adaptation Medium, PM = Production Medium, FMA = Feed Medium A, FMB = Feed Medium B.

Methods

Cell Line and Seed Train Culture

A CHO-DG44 cell line producing a recombinant IgG1 was used in this study. For the seed train, cells were thawed in chemically defined media and cultured in shake flasks in batch mode at 37 °C with 8% CO_2 and a shaking speed of 120 rpm for three passages before inoculation. Production cultures were directly inoculated without prior adaptation into the respective medium.

Culture Media

Table 1 lists the media and feeds, the performance of which was compared to the reference media and feed. For all cultures, 4 mM GlutaMAX™ was added to the basal media.

Offline Analysis

VCD and viability were measured by trypan blue and the Countess™ 3 Automated Cell Counter (ThermoFisher). Glucose was measured using a blood glucose meter following 2 × dilution whenever necessary. IgG1 titer was measured using ELISA.

Experiment 1 | First Media Screening in Shake Flasks

Production cultures were inoculated directly into 30 mL 4Cell® CHO Medium TCX6D, 4Cell® CHO Medium TC-42, 4Cell® CHOlean Medium, 4Cell® XtraCHO PM, or reference medium with a seeding density of 0.3×10^6 cells/mL. Cells were cultured in shake flasks at 37 °C, 8% CO_2 atmosphere, and 120 rpm shaking speed. 4Cell® Basic Feed, 4Cell® XtraCHO FMA and FMB, or reference feed were added as shown in Table 2. The feeding strategies for Sartorius media were determined in accordance with the instructions for use. Glucose solution (450 g/L) was added to maintain a target concentration of 4 g/L whenever the concentration dropped below 2 g/L from day 3 to day 14. Glucose was measured daily from day 3 until day 13 before feed addition.

Table 2: *Feeding Strategy Used During the First Media Screening in Shake Flasks (Experiment 1)*

Basal Medium	Feed	Initial Volume [%]													
		Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13
4Cell® CHO Medium TCX6D	4Cell® Basic Feed				2.0	2.0	3.0	3.0	3.0	3.0	3.0	4.0	4.0	4.0	4.0
4Cell® CHO Medium TC-42	4Cell® Basic Feed				2.0	2.0	3.0	3.0	3.0	3.0	3.0	4.0	4.0	4.0	4.0
4Cell® CHOlean Medium	4Cell® Basic Feed				2.0	2.0	3.0	3.0	3.0	3.0	3.0	4.0	4.0	4.0	4.0
4Cell® XtraCHO PM	4Cell® XtraCHO FMA				2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
	4Cell® XtraCHO FMB				0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
4Cell® XtraCHO PM	4Cell® XtraCHO FMA				4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
	4Cell® XtraCHO FMB				0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
4Cell® XtraCHO PM	4Cell® XtraCHO FMA				6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
	4Cell® XtraCHO FMB				0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Reference	Reference				3.3		3.3		3.3			3.3			

Note. The number indicates the percentage of the initial volume. PM = Production Medium, FMA = Feed Medium A, FMB = Feed Medium B.

Experiment 2 | Second Media Screening in Shake Flasks

Production cultures were inoculated directly into 30 mL 4Cell® CHO Medium TCX6D, 4Cell® CHO Medium TC-42, 4Cell® CHOlean Medium, 4Cell® XtraCHO PM, or reference medium with a seeding density of 0.6×10^6 cells/mL. Cells were cultured in shake flasks at 35 °C, 8% CO_2 atmosphere, and 120 rpm shaking speed.

No feed was added on days 8 and 9. 4Cell® Basic Feed, 4Cell® XtraCHO FMA and FMB, or reference feed were added as shown in Table 3. Glucose was measured every day from day 3 until day 7 and from day 10 until day 13 before feed addition. Glucose solution (450 g/L) was added to maintain a target concentration of 4 g/L whenever the concentration dropped below 2 g/L.

Table 3: *Feeding Strategy Used During the Second Media Screening in Shake Flasks (Experiment 2)*

Basal Medium	Feed	Initial Volume [%]													
		Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13
4Cell® CHO Medium TCX6D	4Cell® Basic Feed				2.0	2.0	3.0	3.0	9.0			4.0	4.0	4.0	4.0
4Cell® CHO Medium TC-42	4Cell® Basic Feed				2.0	2.0	3.0	3.0	9.0			4.0	4.0	4.0	4.0
4Cell® CHOlean Medium	4Cell® Basic Feed				2.0	2.0	3.0	3.0	9.0			4.0	4.0	4.0	4.0
4Cell® XtraCHO PM	4Cell® XtraCHO FMA				2.0	2.0	2.0	2.0	6.0			2.0	2.0	2.0	2.0
	4Cell® XtraCHO FMB				0.2	0.2	0.2	0.2	0.6			0.2	0.2	0.2	0.2
Reference	Reference				3.3		3.3		3.3			3.3			

Note. The number indicates the percentage of the initial volume. PM = Production Medium, FMA = Feed Medium A, FMB = Feed Medium B.

Experiment 3 | pH, 4Cell® Basic Feed, and Cell Density Optimization Run in Ambr® 15

The Ambr® 15, with 24 single-use bioreactor vessels was used to test eighteen conditions as part of a DOE experiment. The remaining six vessels were used for a supplementary experiment to evaluate different basal medium and feed combinations.

DOE Settings

MODDE® DOE software was used to generate a central composite design (Figure 1). A pH range from 6.9 to 7.4, a seeding density from 0.3×10^6 to 0.6×10^6 cells/mL, and a 4Cell® Basic Feed daily addition range of 2% to 4% were evaluated (Table 3). Titer, integrated viable cell density (IVCD), and viability were analyzed as responses. Data analysis and model generation were performed using MODDE®.

Supplemental Experiment Settings

The conditions are shown in Table 4. pH and seeding density were set to the same value as the center points of the DOE experiment.

Bioreactor Settings

All cultures were carried out at the following set points: 40% DO, 700 rpm stirring speed, and 35 °C temperature. pH was controlled using CO_2 and 1 M Na_2CO_3. The dead band for pH was set to ± 0.05. pH recalibration was done using the Ambr® 15 analysis module (built into the Ambr® 15 system) on days 0 and 1 and every two days afterward. CO_2 pH control was initiated on day 0 after pH sensors were recalibrated. The addition of the base solution was enabled only after the pH sensors were recalibrated once more on day 1 to prevent unnecessary base addition due to sensor instability at the beginning of the cell culture.

The seeding density ranged from 0.3×10^6 to 0.6×10^6 cells/mL. Cells were inoculated following centrifugation, and the media was changed to fresh production media. Inoculation and media filling in the Ambr® 15 vessels were done manually before initiating the cell cultures. On average, the measured cell density after inoculation was around 25% lower than the target inoculation viable cell density (VCD). This could be due to the loss of cells due to centrifugation before the inoculation.

4Cell® Basic Feed was added to the relevant cultures every day from day 3 to 14. The reference feed (3.3%) was added to the reference medium on day 3, 4, 5, 6, and 10.

Glucose solution (450 g/L) was added to maintain a target concentration of 4 g/L whenever the concentration dropped below 2 g/L from day 3, 7 – 10, and 14. However, glucose depletion was observed on day 7 for some cultures due to the inability to precisely monitor glucose levels outside of working days.

The initial working volume was 12 mL, reduced to 13 mL whenever the volume exceeded 13 mL before feeding to avoid overflowing. On day 0 (and whenever foam formation exceeded half of the head space of the vessel), 20 µL antifoam (250 × diluted Antifoam C) was added. Offline analysis was performed on 200 µL samples taken on day 0, 3, 7 – 10, and 14.

Figure 1: *Experimental Design for the pH, 4Cell® Basic Feed, and Cell Density Optimization Run in Ambr® 15 (Experiment 3)*

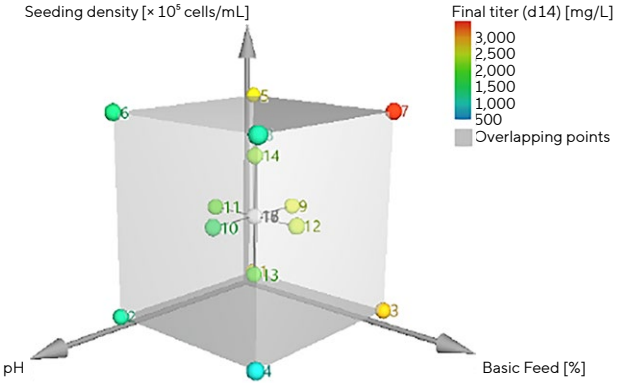


Table 3: *DOE Factors for the pH, 4Cell® Basic Feed, and Cell Density Optimization Run in Ambr® 15 (Experiment 3)*

Factor	Range
pH	6.9 – 7.4
Seeding density	0.3×10^6 – 0.6×10^6 cells/mL
4Cell® Basic Feed	2 – 4%/day, every day from day 3

Table 4: *Supplemental Experiment Settings for the pH, 4Cell® Basic Feed, and Cell Density Optimization Run in Ambr® 15 (Experiment 3)*

Basal Medium	Feed	pH	Seeding Density [1 × 10^6 cells/mL]	Feed [%/day]
4Cell® CHO Medium TCX6D	4Cell® CHO Feed TCX7D	7.15	0.45	1.00
4Cell® CHO Medium TCX6D	4Cell® Basic Feed + 4Cell® XtraCHO FMB	7.15	0.45	3.00 | 0.30
4Cell® CHO Medium TCX6D	4Cell® XtraCHO FMA + 4Cell® XtraCHO FMB	7.15	0.45	3.00 | 0.30
Reference	Reference	7.15	0.45	3.30

Experiment 4 | pH, 4Cell® Basic Feed, and 4Cell® SmartCHO FMB Optimization Run in Ambr®

As in Experiment 3, eighteen conditions were used for the execution of a DOE experiment, and the remaining six vessels were used for a supplementary experiment to evaluate the effect of fresh media replacement at inoculation as well as 4Cell® SmartCHO FMB addition on titer.

DOE Settings

MODDE® DOE software was used to generate a central composite design (Figure 2). A pH range from 6.8 to 7.0, a 4Cell® Basic Feed daily addition range of 3% to 5%, and a 4Cell® SmartCHO FMB daily addition range from 0.2% to 0.6% were evaluated (Table 5). Titer, IVCD, and viability were analyzed as responses. Data analysis and model generation were performed using MODDE®.

Supplemental Experiment Settings

The conditions are shown in Table 6. pH and seeding density were set to the same value as the center points of the DOE experiment.

Bioreactor Settings

All cultures were carried out at the following set points: 40% DO, 700 rpm stirring speed, and 35 °C temperature. pH was controlled using CO_2 and 1 M Na_2CO_3. The pH dead band was set to ± 0.05. pH recalibration and pH control initiation were done as described for Experiment 3.

The seeding density was set to 0.6 × 10^6 cells/mL. Cells were inoculated without centrifugation and the seed train cell culture fluid was added directly to initiate the production cultures. Inoculation and media filling in the Ambr® 15 vessels were done manually before initiating the cell cultures.

4Cell® Basic Feed and 4Cell® SmartCHO FMB were added to the relevant cultures daily from day 3 to 14. The reference feed (3.3%) was added in the reference medium on day 3, 5, 7, and 10. Glucose solution (450 g/L) was added to maintain a target concentration of 6 g/L whenever the concentration dropped below 2 g/L.

The initial working volume was 12 mL, reduced to 13 mL whenever the volume exceeded 13 mL before feeding to avoid overflowing. On day 0 (and whenever foam formation exceeded half of the head space of the vessel), 20 µL antifoam (250 × diluted Antifoam C) was added. Offline analysis was performed on 200 µL samples taken on day 0, 3 – 7, 10, 12, and 14.

Figure 2: *Experimental Design for the pH, 4Cell® Basic Feed, and 4Cell® SmartCHO FMB Optimization Run in Ambr® 15 (Experiment 4)*

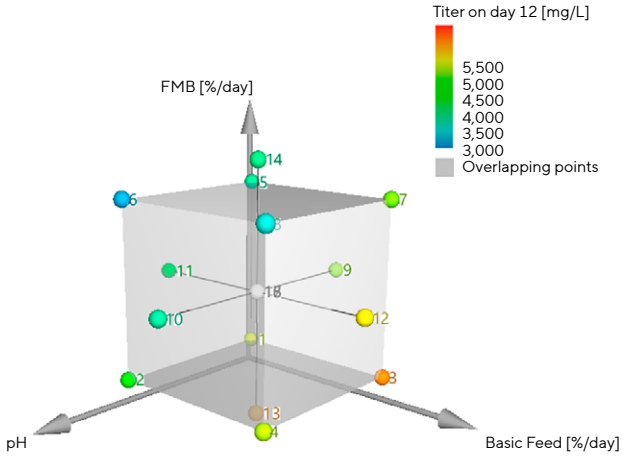


Table 5: *DOE Factors for the pH, 4Cell® Basic Feed, and a 4Cell® SmartCHO FMB Optimization Run in Ambr® 15 (Experiment 4)*

Factor	Range
pH	6.8 – 7.0
4Cell® Basic Feed	3 – 5%/day, every day from day 3
4Cell® SmartCHO FMB	0.2 – 0.6%/day, every day from day 3

Table 6: *Supplemental Experiment Settings for the pH, 4Cell® Basic Feed, and a 4Cell® SmartCHO FMB Optimization Run in Ambr® 15 (Experiment 4)*

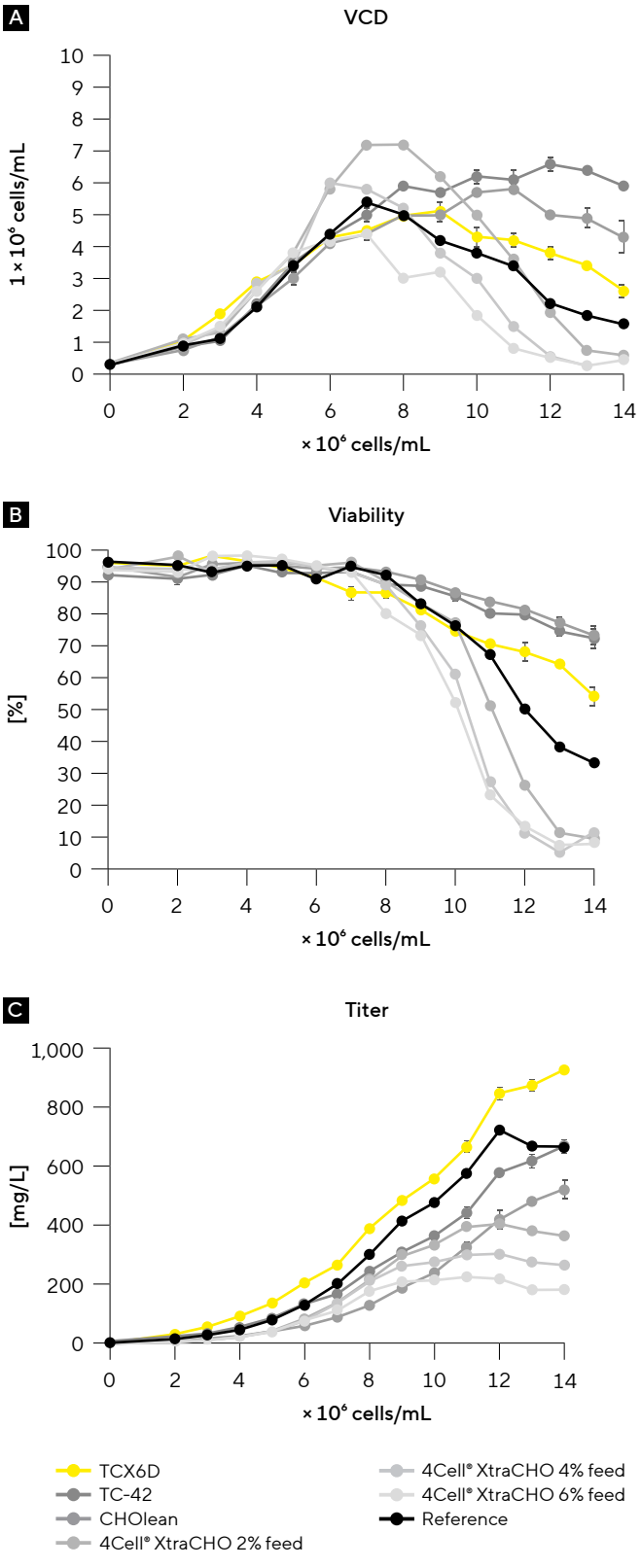
Basal Medium	Feed	Fresh Media Replacement	pH	Feed [%/day]
4Cell® CHO Medium TCX6D	4Cell® Basic Feed	Yes	6.9	4.0
4Cell® CHO Medium TCX6D	4Cell® Basic Feed + 4Cell® SmartCHO FMB	Yes	6.9	4.0 | 0.4
Reference	Reference	No	6.9	3.3
Reference	Reference	Yes	6.9	3.3

Results

Experiment 1 | First Media Screening in Shake Flasks
Initially, we tested five different basal media and feed combinations in shake flasks to select the best-performing media. The 2%/day 4Cell® XtraCHO Feed culture showed the highest VCD (Figure 3A). The viability of all 4Cell® XtraCHO cultures drastically dropped after day 8 (Figure 3B), resulting in low titers at the end of the experiment.

4Cell® CHO Medium TCX6D culture achieved the highest titer on day 14 (Figure 3C) despite the lower VCD compared to other media cultures. The highest titer, including the reference culture, was ~1 g/L, lower than the cell line's historical best performance. Since this experiment was done following the instructions for use for Sartorius 4Cell® XtraCHO media, it is possible that the conditions, including temperature and seeding density, were not appropriate for this cell line. Therefore, the next experiment was conducted using conditions our collaborator previously established to perform well for this cell line. Since the low feed concentration culture performed well in 4Cell® XtraCHO samples, only the 4Cell® XtraCHO Feed 2%/day culture was repeated in the next experiment.

Figure 3: *Performance Comparison of Different Media Screened in Shake Flasks (Experiment 1)*



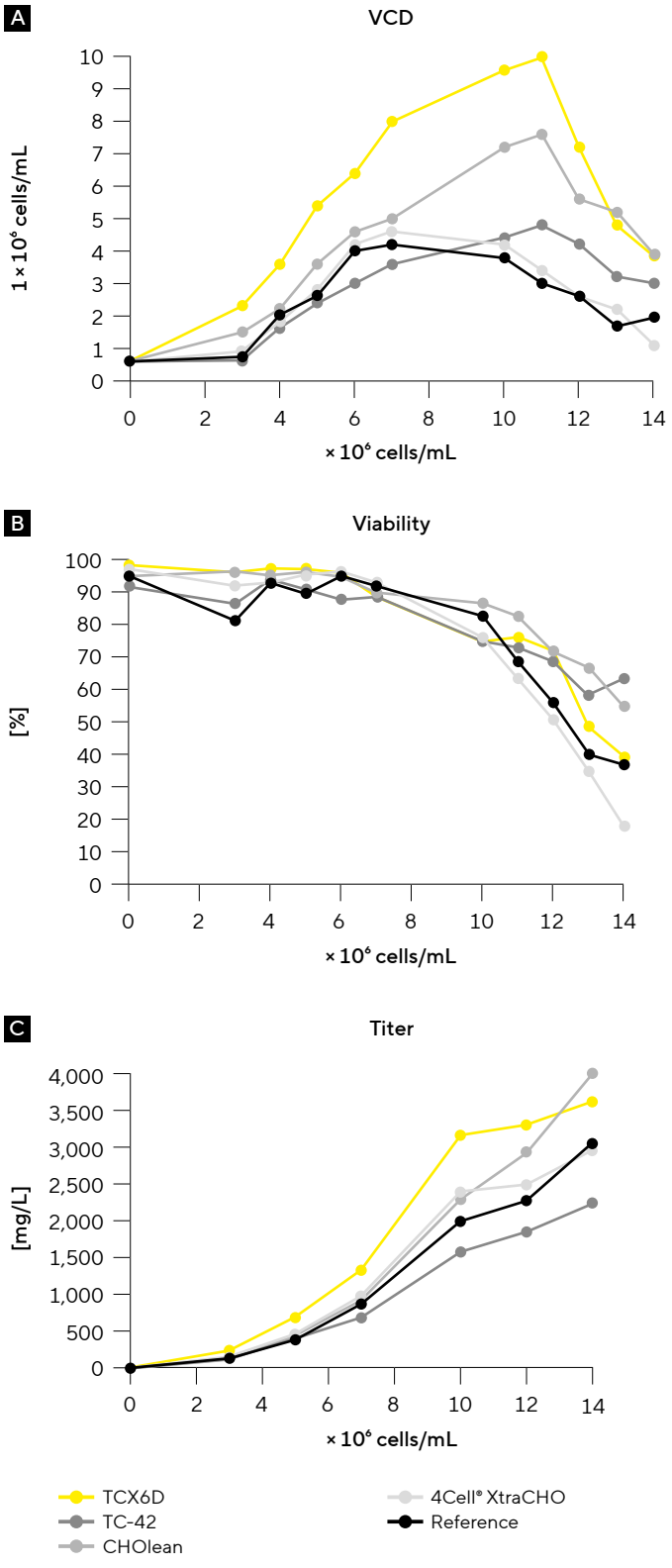
Note. **(A)** Viable cell density (VCD), **(B)** Viability measured by trypan blue cell counting, and **(C)** Titer measured by ELISA. 4Cell® CHO Medium TCX6D, 4Cell® CHO Medium TC-42, and 4Cell® CHOlean Medium cultures were evaluated in duplicate.

Experiment 2 | Second Media Screening in Shake Flasks
In Experiment 2, the conditions were kept consistent with Experiment 1, except that the seeding density was changed from 0.3×10^6 to 0.6×10^6 cells/mL and the temperature from 37 to 35 °C.

4Cell® CHO Medium TCX6D culture achieved the highest VCD (Figure 4A) and the highest titer (Figure 4C) until day 11 when the viability started to drop for all cultures (Figure 4B). The titer of 4Cell® CHOlean Medium culture increased from day 12 to 14 and exceeded the titer of 4Cell® CHO Medium TCX6D culture on day 14. Considering the significant decrease in VCD and viability from day 12 to 14, the titer increase is unexpected. The previous experiment results also demonstrated that 4Cell® CHO Medium TCX6D cultures performed better than 4Cell® CHOlean Medium cultures in terms of titer (1.8-fold higher, Figure 3C). Considering these findings, we conclude that 4Cell® CHO Medium TCX6D was the best-performing media for this cell line.

Altering the culture conditions helped 4Cell® CHO Medium TCX6D culture reach a titer of 3.5 g/L. However, none of the shake flask cultures reached the goal titer of 5 g/L. Furthermore, the final viability was 40%, which is relatively low and may present an issue during purification. The limited control of some process parameters, e.g., pH and DO, is most likely the cause for the early drop in viability (Figures 3B and 4B). Therefore, subsequent experiments were conducted in microbioreactors where cell culture conditions could be controlled and evaluated.

Figure 4: *Performance Comparison of Different Media Screened in Shake Flasks (Experiment 2)*



Note. **(A)** Viable cell density (VCD) and **(B)** Viability measured by trypan blue cell counting, **(C)** Titer measured by ELISA.

Experiment 3 | pH, 4Cell® Basic Feed, and Cell Density Optimization Run in Ambr® 15

We conducted two optimization DOE experiments in 24-way Ambr® 15. To improve the titer, pH, seeding density, and 4Cell® Basic Feed daily addition were selected as factors for optimization, as these parameters are known to significantly impact productivity. A central composite design was chosen because it is an orthogonal design that allows the simultaneous optimization of all factors and can provide insights into potential interactions. For Experiment 3, star points inside the design space were chosen because the lower settings of the same design with star points outside of the design space would not be feasible for some of the factors evaluated in the experiment.

Replicate plots (Figure 5A) display the measured response values for all samples evaluated. The blue squares indicate replicate samples at the center point conditions of the DOE. These plots show that different factor settings have a significant impact on each of the responses and that there is good reproducibility for vessels run under the same conditions. Figure 5B shows the observed vs. predicted plots that allow the evaluation of the model's accuracy graphically and to visualize outliers. Most plots were on the line, representing good predictability in all responses.

Figure 5C demonstrates the quality of the generated models by measuring the following values: coefficient of determination R^2, adjusted coefficient of determination Q^2, model validity, and reproducibility. R^2 and Q^2 should be as close to 1 as possible and as close to each other as possible for good models. Q^2 is a measure of the capability of the model to predict future results. A model validity of more than 0.25 is considered good. According to Figures 5A–C, good models were created for all evaluated responses. Although a few outliers were observed, the data was included in the model because they did not significantly affect the models, and we could not identify the cause of the discrepancy.

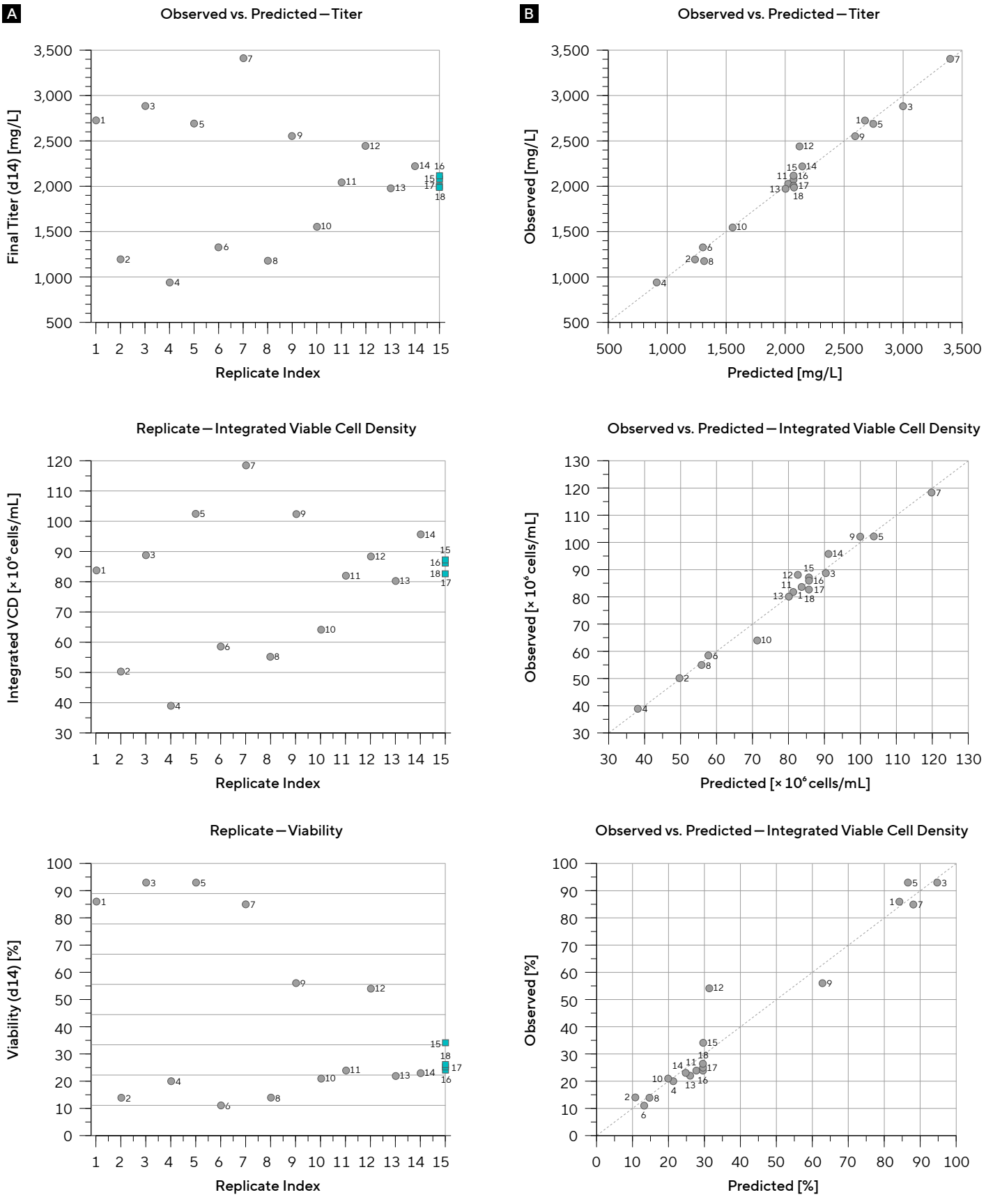
Figures 5D–F display contour plots of each response: red indicates a high value, and blue indicates a low value. Figure 5D shows that high seeding density, high 4Cell® Basic Feed concentration, and low pH resulted in high titer. It also demonstrates that the best conditions were at the edge of the design space. Low pH also resulted in high IVCD (Figure 5E) and high viability (Figure 5F).

Supplemental experiments were also conducted to test the performance of different feeds with 4Cell® CHO Medium TCX6D. As shown in Figure 5G, the addition of 4Cell® XtraCHO FMB to 4Cell® Basic Feed resulted in a 10–15% increase in final titer. In contrast, other media and feed combinations underperformed compared to 4Cell® Basic Feed-only cultures. The predicted highest titer of 4Cell® CHO Medium TCX6D and 4Cell® Basic Feed culture was higher than that of reference media cultures (Figure 5H). These results suggest that 4Cell® XtraCHO FMB addition and further optimization of cell culture conditions might result in higher productivity. Therefore, a confirmation run and further optimization of process parameters and feeding strategy were conducted in a subsequent experiment.

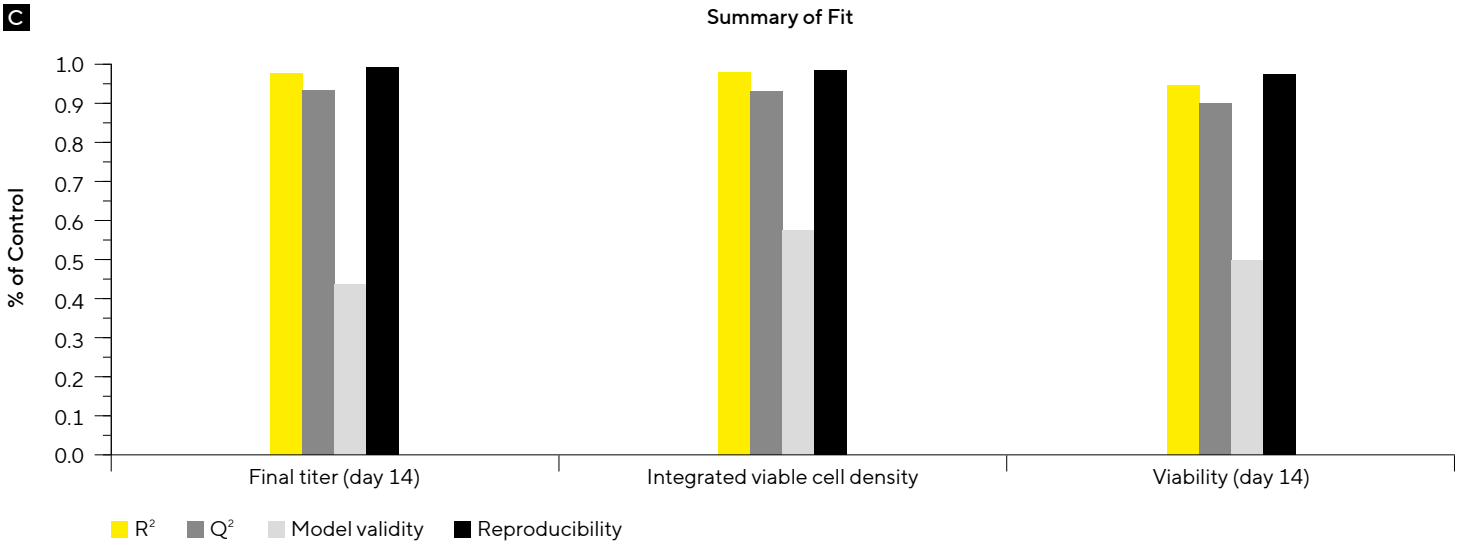
A lower inoculation VCD than the theoretical value (around 25% less on average) was also observed after inoculation. This was assumed to be due to centrifugation, as all cultures were centrifuged and media replaced before inoculation. Therefore, cell inoculation without centrifugation was used in the next experiment.

Figure 5: *DOE Analysis Results From MODDE® and Supplemental Experiment Outcomes for pH, 4Cell® Basic Feed, and Cell Density Optimization in Ambr® 15 (Experiment 3)*

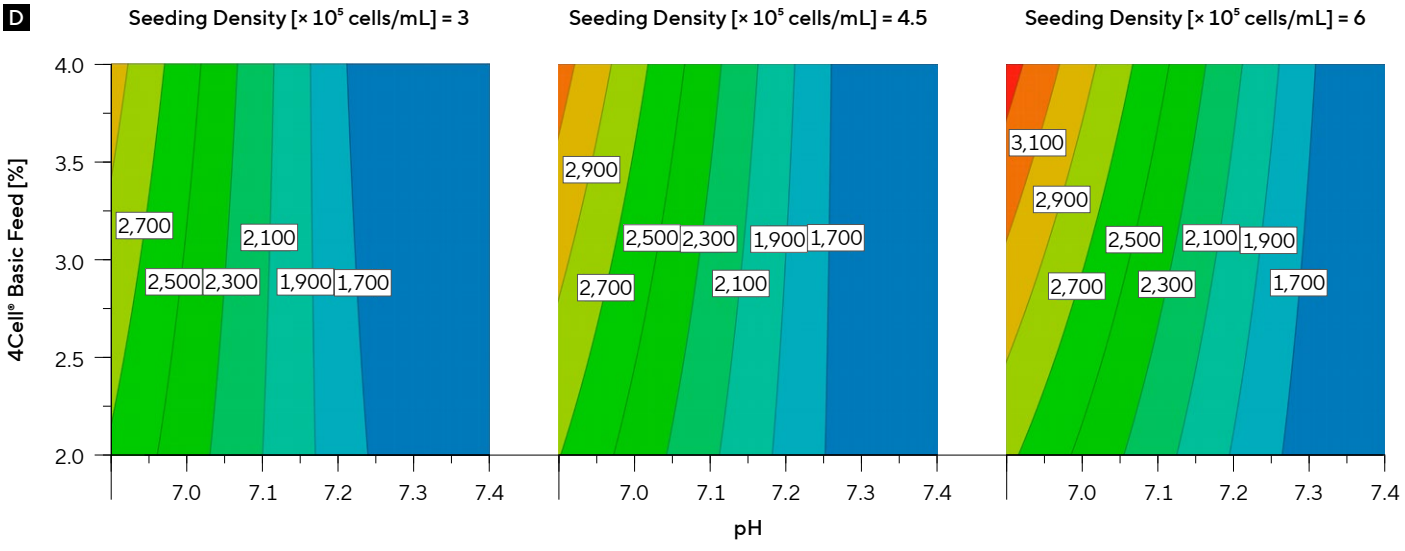
Note. **(A)** Replicate indexes, **(B)** Observed vs. predicted plots, **(C)** Summary of fit, **(D – F)** Contour plots, **(G)** Titer comparison among different media and feed combinations, **(H)** Titer comparison between the reference media culture and 4Cell® CHO Medium TCX6D culture with predicted best conditions.



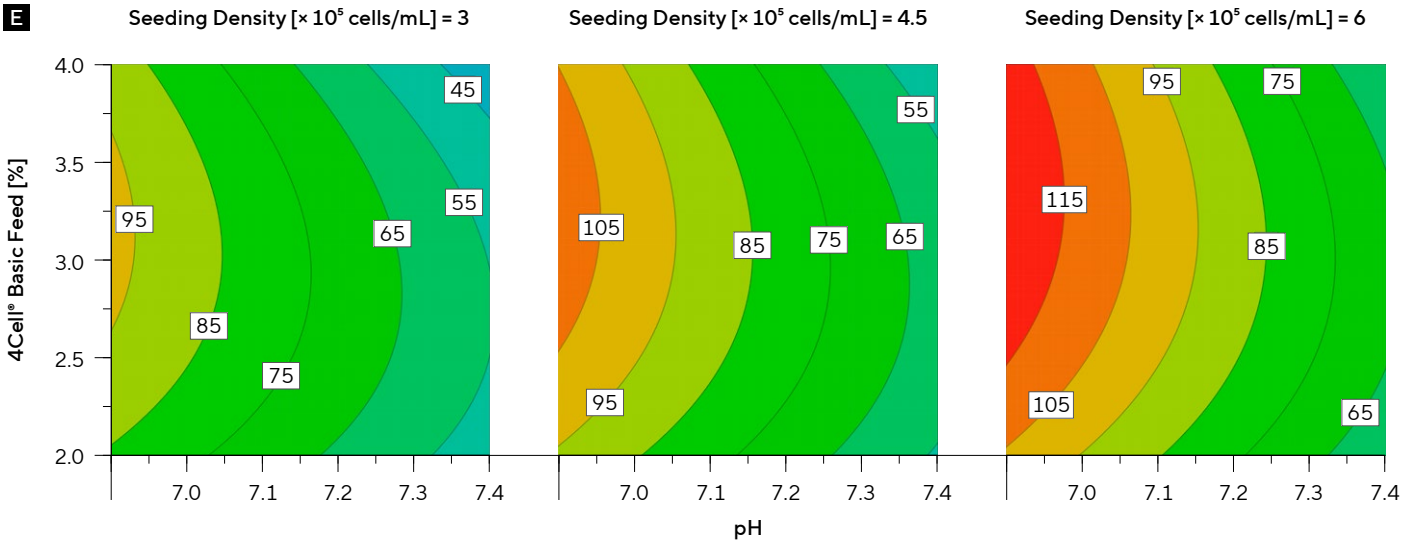
C

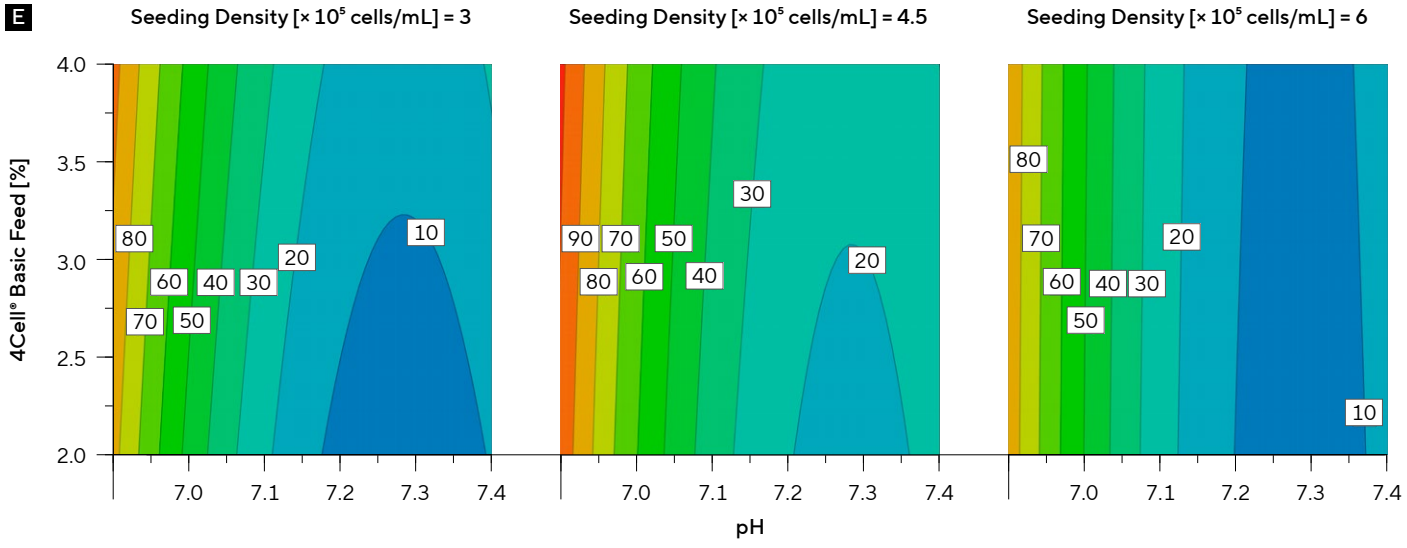

Summary of Fit
% of Control
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Final titer (day 14)
Integrated viable cell density
Viability (day 14)
R²
Q²
Model validity
Reproducibility

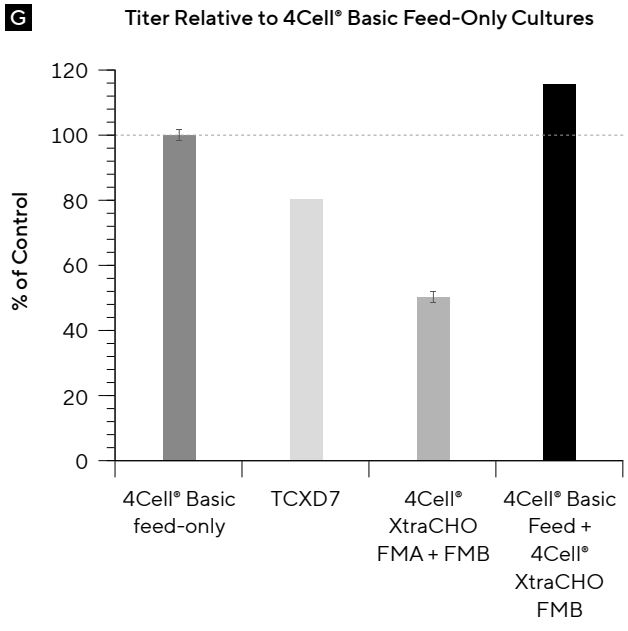
D

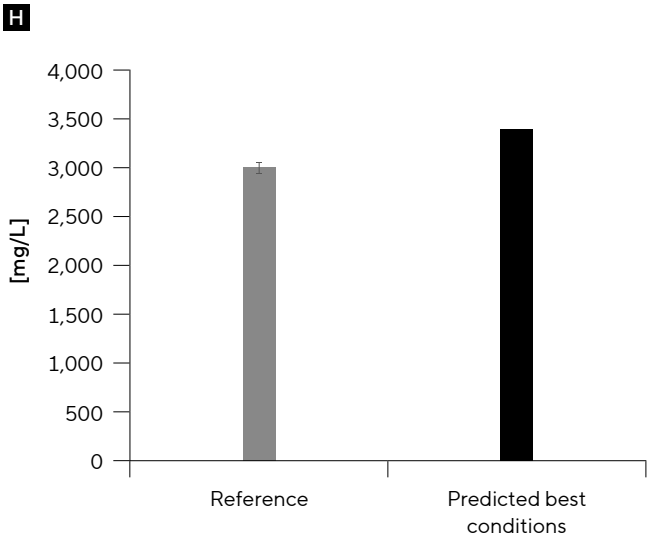

Seeding Density [× 10⁵ cells/mL] = 3
Seeding Density [× 10⁵ cells/mL] = 4.5
Seeding Density [× 10⁵ cells/mL] = 6
4Cell® Basic Feed [%]
4.0
3.5
3.0
2.5
2.0
2,700
2,100
2,500
2,300
1,900
1,700
2,900
3,100
7.0
7.1
7.2
7.3
7.4
pH

E


Seeding Density [× 10⁵ cells/mL] = 3
Seeding Density [× 10⁵ cells/mL] = 4.5
Seeding Density [× 10⁵ cells/mL] = 6
4Cell® Basic Feed [%]
4.0
3.5
3.0
2.5
2.0
45
55
65
75
85
95
105
115
7.0
7.1
7.2
7.3
7.4
pH


E
Seeding Density [× 10⁵ cells/mL] = 3
Seeding Density [× 10⁵ cells/mL] = 4.5
Seeding Density [× 10⁵ cells/mL] = 6
4Cell® Basic Feed [%]
4.0
3.5
3.0
2.5
2.0
80
60
40
30
20
70
50
10
90
70
50
80
60
40
30
20
80
70
60
40
30
20
50
10
7.0
7.1
7.2
7.3
7.4
pH


G
Titer Relative to 4Cell® Basic Feed-Only Cultures
% of Control
120
100
80
60
40
20
0
4Cell® Basic feed-only
TCXD7
4Cell® XtraCHO FMA + FMB
4Cell® Basic Feed + 4Cell® XtraCHO FMB


H
[mg/L]
4,000
3,500
3,000
2,500
2,000
1,500
1,000
500
0
Reference
Predicted best conditions

Experiment 4 | pH, Basic Feed, and a 4Cell® SmartCHO FMB Optimization Run in Ambr® 15

Based on the previous results, pH, 4Cell® Basic Feed, and 4Cell® SmartCHO FMB addition were chosen as factors in the second DOE experiment. 4Cell® SmartCHO was launched as the newest version of the 4Cell® XtraCHO series, and 4Cell® SmartCHO FMB was used in Experiment 4. The range settings for pH and 4Cell® Basic Feed daily concentration were also shifted to lower and higher, respectively, based on the results obtained in Experiment 3.

In Experiment 4, no significant titer improvement was observed after day 12. However, the viability of almost all cultures declined. Furthermore, the combination of low pH and high 4Cell® SmartCHO FMB concentration resulted in precipitation in some cultures on day 14. Therefore, data from day 12 was used to evaluate this experiment.

In Experiment 4, an overall improvement in titer was observed compared to Experiment 3, even for similar culture conditions. A potential explanation of this result could be better control of inoculation VCD and glucose addition. The VCD measured immediately after inoculation was close to the target theoretical inoculation VCD, and no glucose depletion was observed in all cultures during the process.

Some deviations in the replicate samples (Figure 6A, blue squares) and outliers were observed, especially in the viability graph (Figure 6B). Nevertheless, since the quality of the titer model was good and we could not investigate the cause for these discrepancies (Figure 6C), the data was analyzed without any sample exclusions.

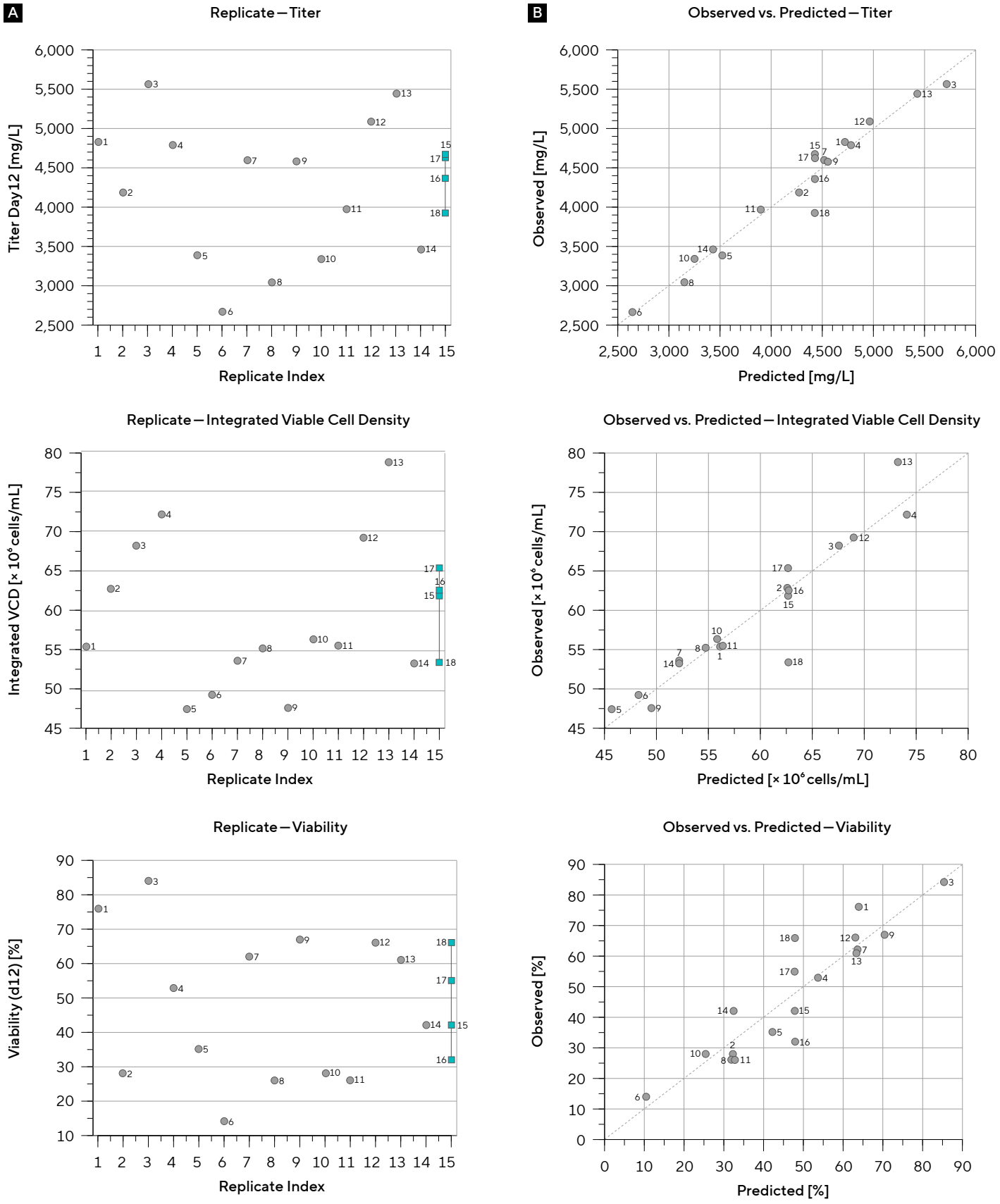
As shown in Figure 6D – F, a low 4Cell® SmartCHO FMB concentration led to high titer, IVCD, and high viability. A high 4Cell® Basic Feed concentration also resulted in high titer, IVCD, and viability. The optimal pH setpoint for the titer was around 6.8 (Figure 6D).

Sweet spot analysis shows the area of design space where titers > 5 g/L and viability > 70% can be achieved (Figure 6G, green). Finally, the best culture conditions using 4Cell® CHO Medium TCX6D media along with 4Cell® Basic Feed and 4Cell® SmartCHO FMB were identified to be pH 6.82, 5.03% 4Cell® Basic Feed, and 0.15% 4Cell® SmartCHO FMB (Figure 6H). With these conditions, the predicted final titer on day 12 was 5.8 g/L. The titer was 1.5 times higher than historical champion data.

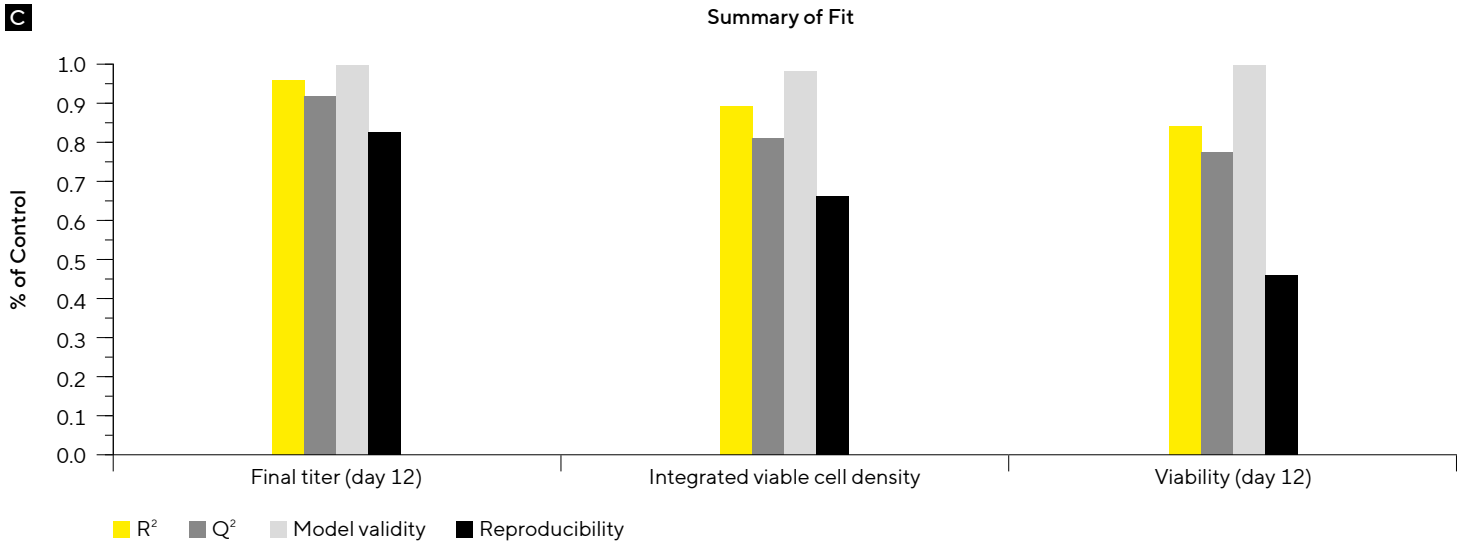
The supplemental experiments were to collect data required to compare the results from Experiments 3 and 4. Since the main difference was media replacement at inoculation, the effect was evaluated for 4Cell® CHO Medium TCX6D and reference media cultures. Figures 6I and J show that media replacement had a positive effect on productivity in both media cultures. Furthermore, Figure 6K demonstrates that 4Cell® SmartCHO FMB addition had a positive impact on titer, consistent with the results obtained from Experiment 3. However, as Figure 6D shows, 4Cell® SmartCHO FMB concentrations higher than 0.2%/day had a negative impact on productivity. In conclusion, the predicted maximal titer was 5.8 g/L (1.7-fold higher compared to the highest titer in Experiment 2), and it was achieved in 12 days in Ambr® 15 vs. 14 days in shake flasks (Figure 6L). The final viability was also improved and expected to be around 85%, reducing the initial material's impurity content and simplifying its purification.

Figure 6: *DOE Analysis Results from MODDE® and Supplemental Experiment Outcomes for pH, 4Cell® Basic Feed, and a 4Cell® SmartCHO FMB Optimization in Ambr® 15 (Experiment 4)*

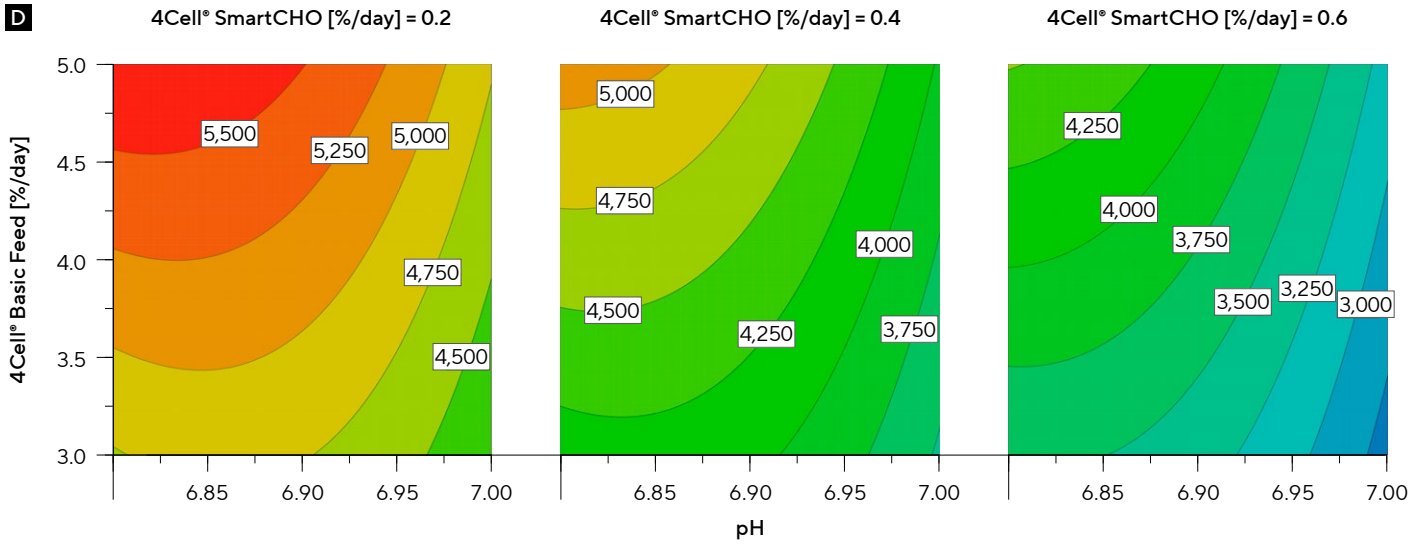
Note. **(A)** Replicate indexes, **(B)** Observed vs. predicted plots, **(C)** Summary of fit, **(D – F)** Contour plots, **(G)** Sweet spot analysis, **(H)** Probability of failure, **(I, J)** Media replacement effect on the titer of 4Cell® CHO Medium TCX6D and reference media cultures, **(K)** 4Cell® SmartCHO FMB effect on the titer of 4Cell® CHO Medium TCX6D cultures. **(L)** Titer and viability comparison between the best conditions from Experiments 2 and 4.



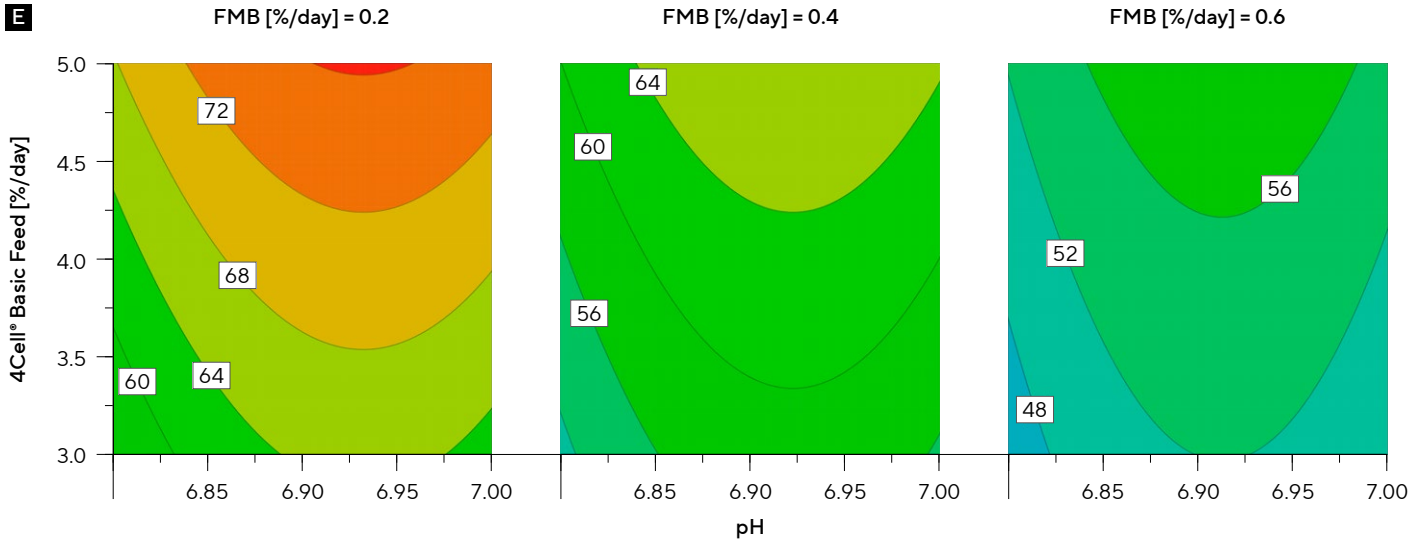
C


Summary of Fit
% of Control
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Final titer (day 12)
Integrated viable cell density
Viability (day 12)
R²
Q²
Model validity
Reproducibility

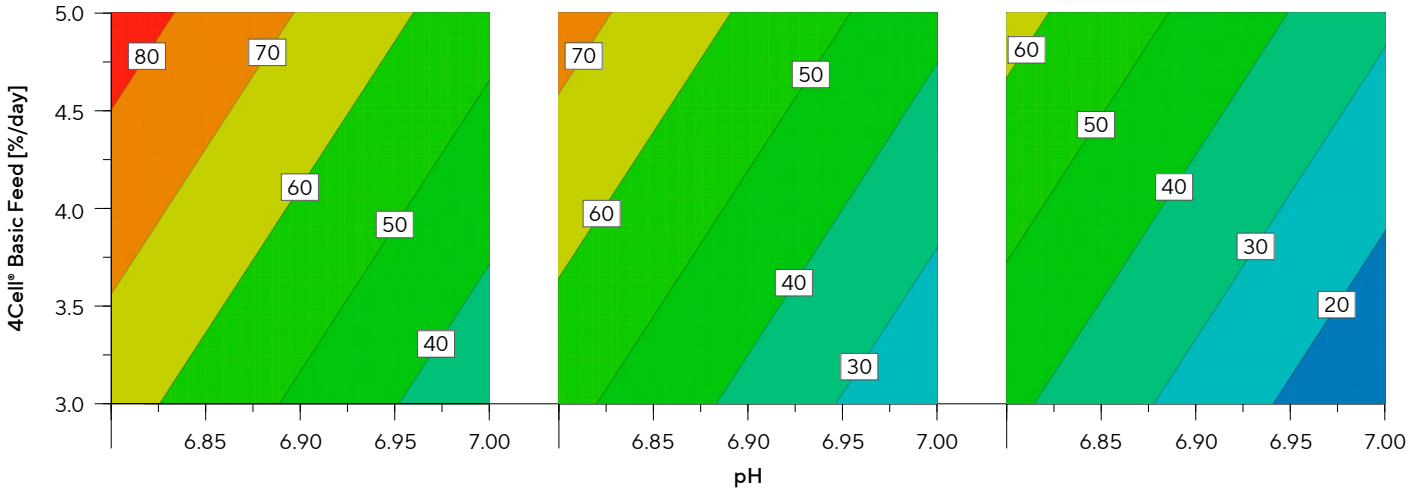
D


4Cell® SmartCHO [%/day] = 0.2
4Cell® SmartCHO [%/day] = 0.4
4Cell® SmartCHO [%/day] = 0.6
4Cell® Basic Feed [%/day]
5.0
4.5
4.0
3.5
3.0
5,500
5,250
5,000
4,750
4,500
5,000
4,750
4,500
4,250
4,000
3,750
4,250
4,000
3,750
3,500
3,250
3,000
6.85
6.90
6.95
7.00
pH

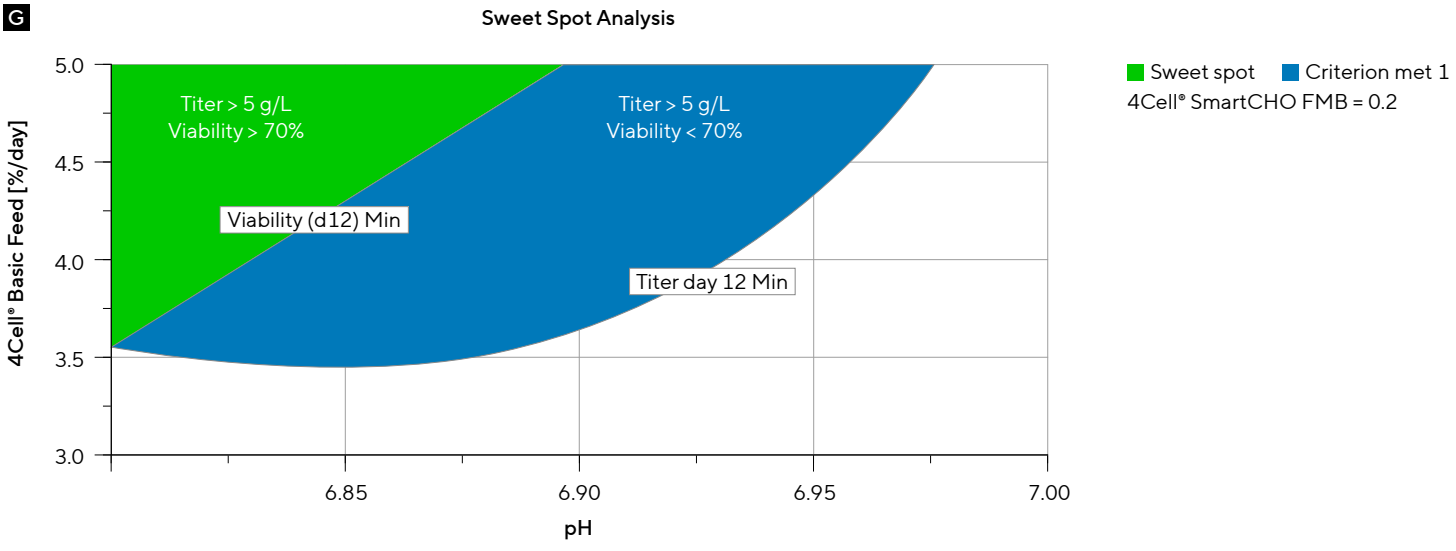
E


FMB [%/day] = 0.2
FMB [%/day] = 0.4
FMB [%/day] = 0.6
4Cell® Basic Feed [%/day]
5.0
4.5
4.0
3.5
3.0
72
68
64
60
64
60
56
56
52
48
6.85
6.90
6.95
7.00
pH

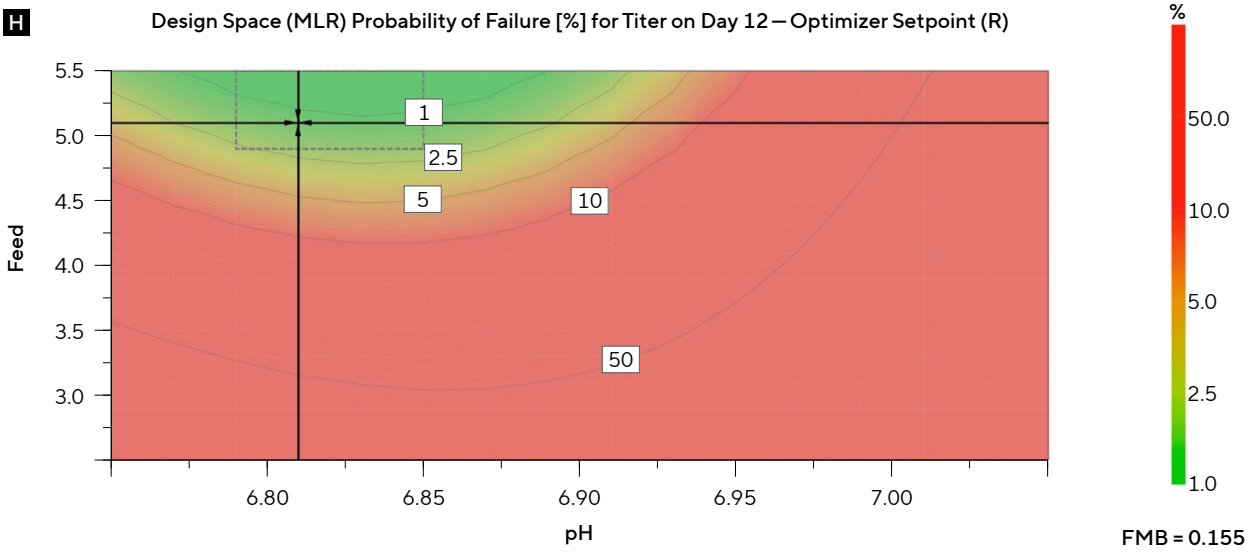
F



G

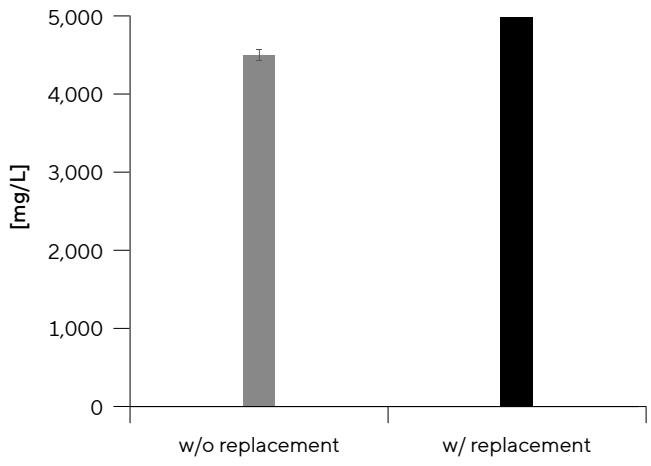


H

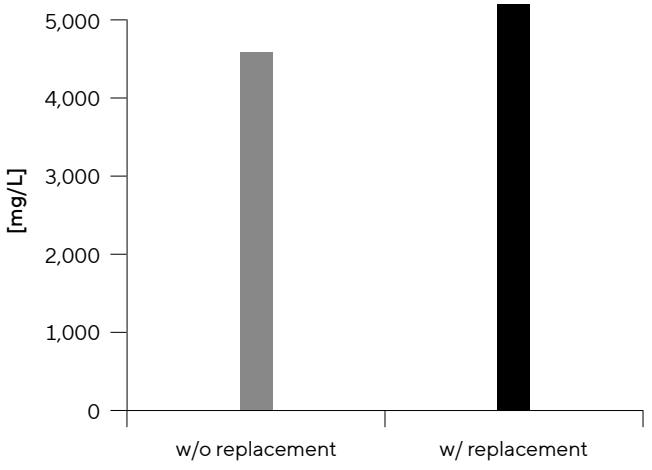


No distributation on factors. Interval = 0.95 Tolerance Acceptance limit = 5%.

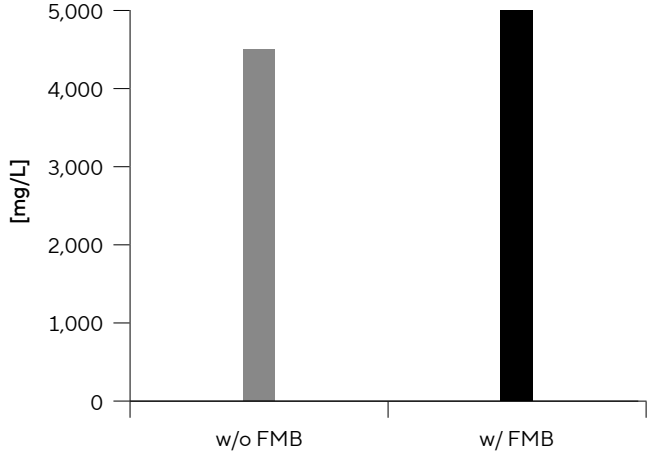
I


Media Replacement Effect
on TCX6D Culture Titers
[mg/L]
5,000
4,000
3,000
2,000
1,000
0
w/o replacement
w/ replacement

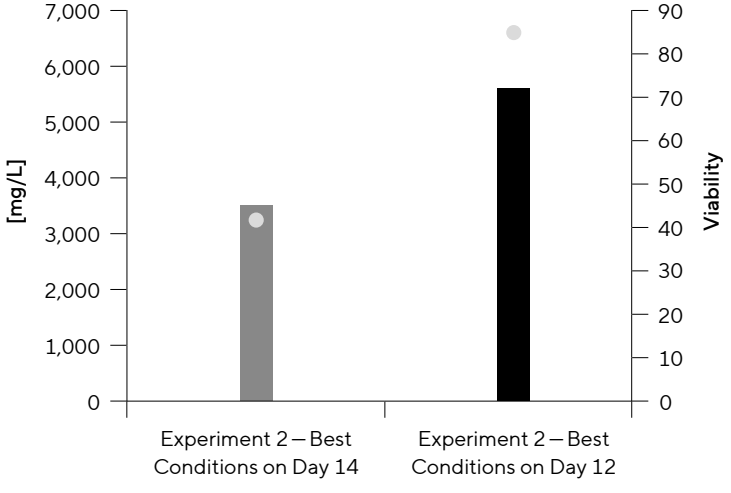
J


Media Replacement Effect
on Reference Culture Titers
[mg/L]
5,000
4,000
3,000
2,000
1,000
0
w/o replacement
w/ replacement

K


4Cell® SmartCHO FMB Effect on Titer
[mg/L]
5,000
4,000
3,000
2,000
1,000
0
w/o FMB
w/ FMB

L


Titer and Viability Comparison
[mg/L]
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
Viability
90
80
70
60
50
40
30
20
10
0
Experiment 2 – Best
Conditions on Day 14
Experiment 2 – Best
Conditions on Day 12

Discussion

In this study, we initially screened multiple media in shake flasks, and 4Cell® CHO Medium TCX6D was chosen as the best-performing media. The main difference between 4Cell® CHO Medium TCX6D and other Sartorius media is that it contains a growth factor, which might have had a positive impact on this cell line.

It is also noteworthy that a companion feed does not always give the best results. 4Cell® SmartCHO FMB addition to 4Cell® Basic Feed increased the final titer (Figure 5G). 4Cell® SmartCHO FMB is one of the companion feeds of 4Cell® SmartCHO that is usually used with 10% volume of the other companion feed, 4Cell® SmartCHO FMA. In this study, the increase in titer with the addition of 4Cell® SmartCHO FMB to 4Cell® Basic Feed appeared to be a result of improved cell-specific productivity, as cell growth curves and final viability of Basic Feed with 4Cell® SmartCHO FMB cultures were similar to those of 4Cell® Basic Feed-only control cultures (data not shown).

Following the process optimization runs, the conditions predicted to achieve a titer of 5.8 g/L were identified. Since the best conditions of 4Cell® Basic Feed and 4Cell® SmartCHO FMB were at the edge of the design space, higher 4Cell® Basic Feed and lower 4Cell® SmartCHO FMB concentrations could be further optimized in future experiments.

High inoculation fed-batch cultures may also improve the final titer, as the results indicate that high seeding density led to increased titer (Figure 5D) while spent media carryover had a negative impact on titer (Figure 6I). Based on these results, N-1 perfusion could be a valuable process intensification approach.

Conclusion

This study aimed to develop a CHO cell culture process that produced an IgG1 titer of > 5 g/L. This goal was achieved by screening the best-performing media in shake flasks and subsequently optimizing the process in the Ambr® 15.

4Cell® CHO Medium TCX6D was selected as the best-performing basal medium, achieving a 1.2-fold higher titer than the reference culture in shake flasks. Seeding density, pH, and feeding strategy were optimized in the Ambr® 15 by executing two consecutive DOE experiments generated in MODDE®. The best feeds were identified as 4Cell® Basic Feed and 4Cell® SmartCHO FMB (5%/day and 0.15%/day, respectively, from day 3 – 12). A seeding density of 6×10^5 cells/mL and pH 6.8 were determined to be the optimal culture conditions. The predicted final titer for these conditions was 5.8 g/L, 1.7-fold higher than the non-optimized shake flask cultures (Figure 6L).

Furthermore, the process duration was shortened from 14 to 12 days, resulting in a similar final titer but higher final viability. In addition, the best performing 4Cell® CHO Medium TCX6D culture in the Ambr® 15 showed a 1.3-fold higher titer than the Ambr® 15 reference media culture.

This case study shows that Sartorius cell culture technologies can save valuable resources and shorten process development timelines. All experiments included in this study were conducted within 3 months. This demonstrates that the combination of the comprehensive CHO media portfolio, the automated high throughput bioreactor Ambr® 15, and the DOE software MODDE® can significantly accelerate the early phase of process development.

References

1. Kim, J. Y., Kim, Y.-G., & Lee, G. M. (2012). CHO cells in biotechnology for production of recombinant proteins: Current state and further potential. Applied Microbiology and Biotechnology, 93(3), 917–930. https://doi.org/10.1007/s00253-011-3758-5

2. Combe, M., & Sokolenko, S. (2021). Quantifying the impact of cell culture media on CHO cell growth and protein production. Biotechnology Advances, 50, 107761. https://doi.org/10.1016/j.biotechadv.2021.107761

3. Xu, W.-J., Lin, Y., Mi, C.-L., Pang, J.-Y., & Wang, T.-Y. (2023). Progress in fed-batch culture for recombinant protein production in CHO cells. Applied Microbiology and Biotechnology, 107(4), 1063–1075. https://doi.org/10.1007/s00253-022-12342-x

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

Japan
Sartorius Stedim Japan K.K.
4F, Daiwa Shinagawa North,
1-8-11 Kita-Shinagawa,
Shinagawa-ku, Tokyo 140-0001
Phone +81 3 6478 5201

For more information, visit
sartorius.com

Last updated: 03 | 2025