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A Data-Guided Strategy for Optimizing CHO Media and Feed Combinations With Minimal Experimentation

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Abstract

Optimizing media and feed combinations for CHO cell cultures is essential but often resource intensive. This white paper outlines a data-guided strategy to identify optimal combinations with minimal experimentation. Using controlled small-scale systems and Sartorius 4Cell® media and feeds, the approach integrates glucose-based feeding and spent-media analytics to efficiently evaluate titer, metabolism, and product quality. The framework enables faster, more informed process optimization and smooth scale-up to benchtop bioreactors.

Introduction

In the biopharmaceutical industry, the production of high-quality therapeutic proteins, such as monoclonal antibodies (mAbs), is critically dependent on the choice of culture media and feed strategies. These factors not only influence the mAb titer but also impact the product quality attributes (PQAs),[1,2] including monomer content, charge variants, and glycosylation profiles. Therefore, identifying the optimal medium | feed combination is essential for ensuring both high yield and the desired PQAs.

Traditionally, screening various media and feed combinations is a complex and labor-intensive process.[3] This increases the risk of overlooking suitable combinations due to suboptimal feed strategies. To mitigate this, multiple feed regimes can be tested, which further increases the number of experiments needed. Fixed feed regimes cannot adapt to the dynamic nutrient demand of a cell culture, resulting in over- or underfeeding.[1]

An alternative approach involves calculating the feed volume based on the demand for a lead substrate. Glucose is the preferred lead substrate to control the feed regime because it can be measured easily and at low costs.[2,4,5] If the glucose demand of the culture correlates with the demand for other nutrients like amino acids, this strategy enables dynamic adaptation of the feed to cellular requirements and can improve process performance.[4]

However, if the consumption rate of glucose and other nutrients does not correlate over time, the result may be an imbalanced feed process.[2]

Moreover, conventional screening methods, such as shake flask cultures, are conducted under uncontrolled conditions, which may not accurately reflect the outcomes in a controlled bioreactor environment. As a result, combinations that perform well in shake flasks might fail to deliver the same results in a scaled-up, controlled process.

In this study, we address these challenges by using an Ambr® 15 system to efficiently screen various media (4Cell® CHOlean Medium, 4Cell® CHO Medium TCX6D, TCX10D, and SmartCHO) and feed (4Cell® CHO Feed TCX7D, 4Cell® SmartCHO Feed Medium A [FMA], and 4Cell® SmartCHO Feed Medium B [FMB]) combinations for two CHO cell lines, each expressing an immunoglobulin G (IgG), in fed-batch cultures.

The PQAs of all tested combinations from the Ambr® 15 experiments were analyzed to assess the effect of different media | feed combinations on mAb quality. The combination yielding the highest titer for each cell line was subsequently scaled up to a 2 L Biostat® B bioreactor. This approach allowed us to evaluate IgG titers and monitor changes in PQAs during the cultivation. In addition, glucose served as a lead substrate to determine the feed regime. Additional analyses, such as amino acid and trace element analysis, were employed to evaluate the feed strategy, allowing further refinement.

This study demonstrates a streamlined and automated approach to media and feed screening, providing valuable insights into the impact of these combinations on PQAs and offering a pathway for future optimization. The study also tested the use of the controlled, downscaled Ambr® 15 systems for estimating and optimizing results in a larger-scale bioreactor. In addition, the use of off-the-shelf products enables a simple method transfer, making it broadly accessible.

Materials and Methods

Note on Naming Conventions:
To enhance clarity and readability, simplified product names (e.g., TCX6D, SmartCHO media, FMA) are used throughout the text when referring to Sartorius cell culture media and feeds. Full product names (e.g., 4Cell® CHO Medium TCX6D, 4Cell® SmartCHO Feed Medium A) are retained in key areas for accuracy and completeness.

Analysis of Cell Culture Supernatant

Glucose and lactate levels were analyzed using a standard glucose and lactate analyzer. Osmolality was measured using the Osmomat® 3000 (Gonotec). Ammonium was analyzed by measuring the fluorescence resulting from its reaction with o-phthaldialdehyde and sodium thioglycolate. Amino acids were analyzed via ultra-high-performance liquid chromatography (UHPLC-UV) after derivatization. Trace elements were measured by inductively coupled plasma mass spectrometry (ICP-MS). The final sample titer was analyzed using Protein A affinity chromatography. PQAs, including charge variants and glycosylation profiles, were assessed using a LabChip®. Monomer content was also analyzed by size exclusion chromatography.

Pre-Cultivation of CHO Cells in Shake Flasks

CHO-K1 and CHO-S cell lines were used for the pre-culture phase of subsequent fed-batch experiments in the Ambr® 15 system. Each cell line expressed a different IgG. The pre-culture also served to adapt the cells to the production media, each supplemented with 6 mM glutamine. CHO-S cells were thawed in SmartCHO medium, while CHO-K1 cells were thawed in CHOlean medium. Both cell lines were cultured in 125 mL shake flasks with a working volume of 20 – 50 mL.

Cultures were maintained in an incubator at 37 °C with a shaking speed of 120 rpm and an orbital diameter of 50 mm. CO_2 concentration was set at 5% for all media except for SmartCHO, which required 7.5% CO_2.

Cells were passaged every 3 – 4 days, adjusting a viable cell density of $2 – 3 \times 10^5$ cells/mL after passaging (Table 1). During the second passage, cells were transferred into the various production media through centrifugation and subsequently cultured for seven passages. Viable cell density (VCD) and viability were assessed using a Cedex® Cell Counter (Roche) and the Trypan Blue exclusion method on the day of passaging. Additionally, glucose and lactate levels were periodically measured to ensure that glucose limitation did not occur during the culture period.

Table 1: *Shake Flask Culture Parameters*

Parameter	Value
Temperature	37 °C
Shaking speed	120 rpm
Orbital throw	50 mm
CO_2 (except 4Cell® SmartCHO Production Medium cultures)	5%
CO_2 for 4Cell® SmartCHO Production Medium cultures	7.5%
Shake flask	125 mL, plain
Culture volume	20 – 50 mL
VCD after passaging	$2 – 3 \times 10^5$ cells/mL
Passage rhythm	3 – 4 days

Ambr® 15 Fed-Batch Cultivation

Following pre-cultivation and successful adaptation in the respective production media, CHO-K1 and CHO-S cells were cultured in various media and feed combinations (Table 2) in fed-batch mode using a second-generation Ambr® 15 system equipped with an integrated analysis module and Vi-CELL® XR (Beckman Coulter). Experiments were performed in duplicate. One day prior to inoculation, vessels were installed in the Ambr® 15 system and filled with 11 mL of the designated production media: CHOlean, TCX6D, TCX10D, and SmartCHO. The vessels were equilibrated to 36.8 °C, and the stirring speed was set to 900 rpm using the "Up-Stir" setting. After 2 hours and 30 minutes, dissolved oxygen (DO) and pH control were initiated, with the DO set point at 40% and the upper pH limit at 7.1. A nitrogen flow of 0.1 mL/min was established. From Day 0 onward, pH calibration was performed daily via the integrated analysis module, setting the pH target to 7.0 and the lower limit 6.9, using 500 mM sodium bicarbonate as a base.

Cells from the pre-culture were inoculated after seven passages in the respective media into the Ambr® 15 vessels at a cell density of 3×10^5 cells/mL. The pre-culture cell density was determined using a Cedex® Cell Counter and recorded as the initial cell density for each vessel. The Ambr® 15 system calculated the inoculation volume using the "Add Cells to Inoculate Culture Vessel Equation" program step. Inoculation was performed by providing sufficient culture volume in deep-well plates, and the vessel volume was adjusted to 15 mL with fresh medium post-inoculation.

Throughout the culture period, 20 µL of antifoam was added daily. Cell density and viability were assessed using the integrated Vi-CELL® XR immediately after inoculation and daily thereafter. Glucose and lactate levels were analyzed daily before feed addition, and osmolality was measured at additional time points. Process samples for further analyses, such as amino acid analysis, were collected on various days.

The feeding strategy was based on glucose as the lead substrate, starting from Day 2. Using the "Add Liquid To Culture Vessel Concentration" program step, target and trigger values were defined as specified in a previous white paper.[6] Briefly, when the residual glucose concentration dropped below the trigger value, feed addition was initiated. The feed volume was calculated to reach the defined glucose target value based on the glucose concentration in the feed solution. Trigger and target values are detailed in Figures 7 and 15. When FMB was used, its volume was 10% of FMA. When used in combination with TCX7D feed, FMB was 7.8% of the TCX7D feed volume.

A temperature shift to 34 °C was performed on Day 6. An overview of the cultivation parameters is provided in Table 3. The cultivation was completed after 14 days. The final samples were assessed for titer and PQAs, including charge variants, glycosylation profile, and monomer content.

Table 2: *Media and Feed Combinations Tested in Fed-Batch Screening Experiments*

Cell Line	Medium	Feed
CHO-K1	4Cell® CHO Medium TCX6D	4Cell® SmartCHO FMA + 4Cell® SmartCHO FMB
		4Cell® CHO Feed TCX7D
		4Cell® CHO Feed TCX7D + 4Cell® SmartCHO FMB
	4Cell® CHO-GS Medium TCX10D	4Cell® SmartCHO FMA + 4Cell® SmartCHO FMB
	4Cell®SmartCHO Production Medium	
CHO-S	4Cell® CHOlean Medium	4Cell® SmartCHO FMA + 4Cell® SmartCHO FMB
		4Cell® CHO Feed TCX7D
		4Cell® CHO Feed TCX7D + 4Cell® SmartCHO Feed Medium B (FMB)
	4Cell® CHO Medium TCX6D	4Cell® SmartCHO Feed Medium A (FMA) + 4Cell® SmartCHO Feed Medium B (FMB)
		4Cell® CHO Feed TCX7D
	4Cell® SmartCHO Production Medium	4Cell® SmartCHO Feed Medium A (FMA) + 4Cell® SmartCHO Feed Medium B (FMB)
		4Cell® CHO Feed TCX7D

Table 3: *Ambr® 15 Culture Parameters*

Parameter	Value
Temperature	36.8 °C
Temperature shift	34.0 °C, Day 6
Stirring speed	900 rpm Up-Stir
DO	40%
pH high level	7.1
pH target	7.0
pH low level	6.9
Inoculation VCD	3×10^5 cells/mL
Inoculation volume	15 mL

Cultivation in 2 L Bioreactor

Conditions for the top-performing media and feed combination for each cell line from the Ambr® 15 experiment were scaled up to a 2 L system using a Biostat® B Twin reactor with a 2 L glass vessel equipped with one three-bladed and one Rushton impeller (Table 4). DO was maintained at 40% using the cascade parameters described in Table 5.

The pH set point was 7.0 with a deadband of ± 0.05. Cells were initially cultured at 36.8 °C, with a shift to 34 °C on Day 6. The inoculation cell density was 3×10^5 cells/mL in a volume of 1.2 L. Antifoam was added as needed, and sodium bicarbonate served as a base. Cell density and viability were measured daily using a Cedex® Cell Counter. Glucose, lactate, ammonium concentration, and osmolality were measured daily. Samples for further analyses, such as titer, amino acid profiling, and trace element analysis, were also collected daily.

The feeding regime was calculated based on the Ambr® 15 results, using glucose as the lead substrate. To maintain a constant glucose profile, the feeding strategy was adjusted based on expected glucose consumption and modified during cultivation if necessary. Unlike the Ambr® 15 setup, the daily feed was administered continuously over 24 hours using calibrated external pumps. The volume of FMB was consistently set to 10% of FMA.

The cultivation was completed after 14 days. The titer in the final samples was analyzed, and PQAs, including charge variants, glycosylation profiles, and monomer content, were assessed.

Table 4: *2 L Biostat® B Twin Culture Parameters*

Parameter	Value
Temperature	36.8 °C
Temperature shift	34 °C, Day 6
Stirring speed	168 – 300 rpm
DO	40%
pH target	7.0 ± 0.05
Inoculation VCD	3×10^5 cells/mL
Inoculation Volume	1.2 L

Table 5: *DO Cascade in Biostat® B Twin*

Cascade phase [%]	N_2 flow [ccm]	Air flow [ccm]	O_2 flow [ccm]	Stirring speed [rpm]
0	54	0	0	168
20	0	54	0	168
40	0	36	18	168
60	0	18	36	168
80	0	0	54	168
100	0	0	81	300

Results

Pre-Cultivation of CHO Cells in Shake Flasks

Before the final fed-batch experiments, cells were adapted to the different media in a pre-culture phase. During the pre-culture phase, both CHO-K1 and CHO-S cells were transferred into their respective media after two passages. Both cell lines adapted quickly to all Sartorius media.

For CHO-K1 cells, the highest growth rates were observed in TCX6D and TCX10D, with cell-specific growth rates exceeding 1.0 d^{-1} and maximum cell densities surpassing 11×10^6 cells/mL after four days. Cells cultured in CHOlean and SmartCHO showed maximum growth rates of 0.87 d^{-1} and 0.92 d^{-1}, respectively, with maximum cell densities of 8.6×10^6 cells/mL and 9.4×10^6 cells/mL after four days.

Viability remained above 95% throughout the pre-cultivation period in all Sartorius media after the first passage (Figure 1).

CHO-S cells exhibited comparable performance across different Sartorius media. Cells cultured in CHOlean, TCX6D, and SmartCHO achieved growth rates ranging from 1.0 to 1.1 d^{-1}, with maximum cell densities of 11.7×10^6 cells/mL, 10.6×10^6 cells/mL, and 9.2×10^6 cells/mL, respectively, after four days. Viability remained at or above 95% throughout the pre-cultivation period in all media after the first passage (Figure 2).

Based on this performance, all cultures of both CHO-K1 and CHO-S cells were used in the subsequent Ambr® 15 fed-batch experiment.

Figure 1: *CHO-K1 Viable Cell Density (Solid Lines) and Viability (Dashed Lines) in Pre-Cultures With Four Media*

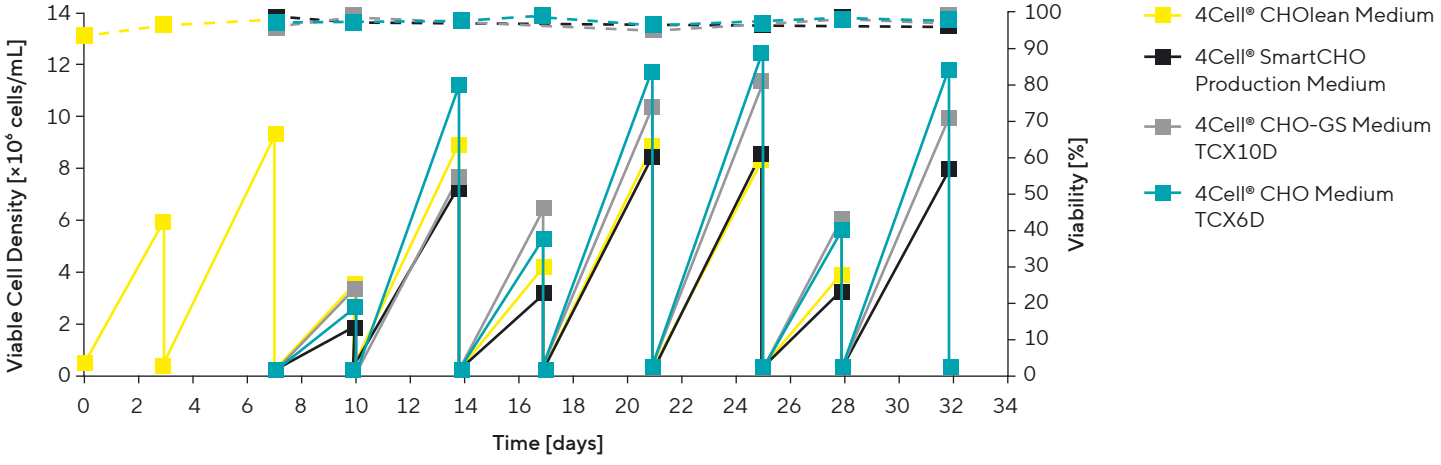
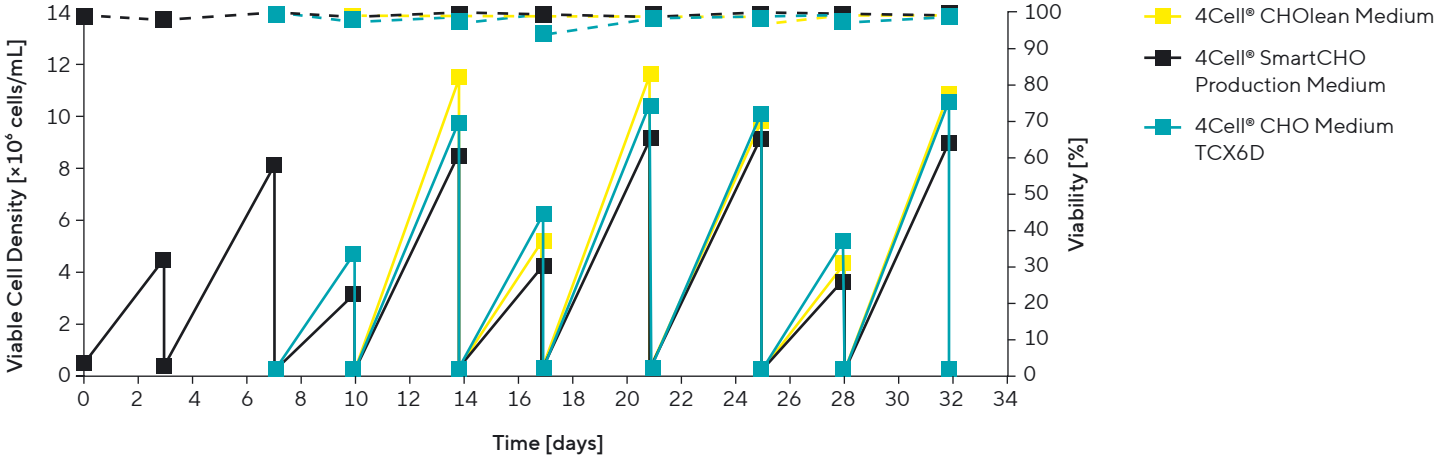


Figure 2: *CHO-S Viable Cell Density (Solid Lines) and Viability (Dashed Lines) in Pre-Cultures With Three Media*



Ambr® 15 Fed-Batch Cultivation of CHO-K1 Cells

To evaluate various media and feed combinations, CHO-K1 cells were cultivated in Ambr® 15 fed-batch processes. Glucose was used as the lead substrate to determine the feed regime.

Cell Growth and Viability
All tested combinations yielded high maximum VCDs, each exceeding 25×10^6 cells/mL. The highest VCDs—38.1×10^6 cells/mL and 40.3×10^6 cells/mL—were achieved using TCX6D with FMA and FMB, corresponding to the highest integral VCD (IVCD) of 290×10^6 cells × day/mL. This was followed by TCX10D with FMA and FMB, which reached a maximum VCD of 35.2×10^6 cells/mL and an IVCD of 255×10^6 cells × day/mL. Other combinations showed slightly lower performances, with IVCDs ranging from 226 to 243×10^6 cells × day/mL. However, VCD measurements were less precise at high cell densities, leading to greater variance between days (Figure 3).

All cultures—except those using TCX6D with TCX7D feed and FMB—maintained high viabilities (≥ 90%) until Day 11. After that point, viability declined in most conditions, with the exception of cultures in SmartCHO with FMA and FMB. Cultures using TCX6D with TCX7D feed and FMB showed the greatest drop in viability, while the remaining combinations exhibited a more moderate decrease to final values between 71% and 82% by Day 14 (Figure 3). Overall, the tested media and feed combinations performed well in terms of CHO-K1 cell growth and viability.

Titer
In terms of titer, FMA and FMB outperformed TCX7D feed, with titers exceeding 2,900 mg/L with all production media. In contrast, no condition using TCX6D with TCX7D feed achieved titers above 2,314 mg/L. The highest average titer—over 3,063 mg/L—was observed when TCX6D was combined with FMA and FMB (Figure 4). However, potential O_2 supply limitations between Days 8 and 12, indicated by DO values dropping below the set point of 40% for several hours, suggested that an even higher titer may have been achievable. Therefore, this combination was chosen for scale-up experiments and subsequent spent media analysis. All tested conditions resulted in a high monomer content of over 96%.

Glycosylation
The glycosylation profile across all CHO-K1 cultures revealed that G0f was the predominant fraction, comprising over 60% of the total glycan content, except for cultures using SmartCHO with FMA and FMB, where G0f accounted for 49%.

All other N-glycans remained below 10%, except for cultures using SmartCHO with FMA and FMB, which showed an 11% G1f representation. The overall presence of mannose structures was low to moderate, ranging from 0.3% to 2.2%. Notably, cultures with SmartCHO and FMA and FMB exhibited a higher representation of galactosylated glycans, specifically G1', G1f, and G2f. Using TCX10D significantly enhanced the G0 fraction compared to TCX6D, with a 2.4-fold increase.

In summary, the choice of production medium had a small influence on the glycan structure. The choice of feed had a minimal impact on the glycosylation profile; however, when TCX7D feed was combined with TCX6D, the G0 fraction increased by a factor of 2.1 (Figure 5). These results demonstrate that although the overall influence was modest, the appropriate selection of media and feed combinations can help guide specific glycan characteristics.

Charge Variants
The choice of media and feeds had a minimal impact on charge heterogeneity in the CHO-K1 cultures (Figure 6). The main peak consistently fell within the range of 52–64% across all experiments, with cultures using SmartCHO and FMA and FMB showing the lowest proportion of the main peak. Notably, the use of TCX7D feed was associated with a modest increase in the proportion of basic variants, rising by up to seven percentage points. Overall, the influence of media and feeds on charge heterogeneity in the CHO-K1 cultures was relatively minor, suggesting that while formulation choices led to some variability, the fundamental characteristics of charge distribution remained largely consistent.

Glucose and Lactate Concentrations
To maintain a feed regime that avoided both overfeeding and nutrient limitations, glucose was used as the lead substrate. Until Day 7, average residual glucose concentrations were higher than during Days 7–14, due to adjustments to lower target and trigger concentrations and the higher cell densities in the second half of the cultivation. Most cultures were not fed on Days 4 and 6, as glucose levels remained above the trigger concentration, preventing overfeeding. After Day 7, most cultures were fed daily, maintaining glucose concentrations between 1-3 g/L (Figure 7).

Lactate concentrations remained non-critical, with no culture exceeding 2.5 g/L. Lactate levels decreased after Day 5 in cultures using TCX6D, TCX10D, or SmartCHO with FMA and FMB, maintaining lactate levels below 0.5 g/L by the end of the cultivation. In contrast, cultures fed with TCX7D feed showed increasing lactate concentrations after 10 days, but remained below 2 g/L (TCX6D with TCX7D feed and FMB) and 1 g/L (in TCX6D with TCX7D feed alone), as shown in Figure 8. The higher lactate concentration observed in TCX6D with TCX7D feed and FMB cultures corresponded to the lower viability in the culture at this time. Overall, the lactate and glucose profiles indicate that the chosen feeding strategy effectively avoided glucose overfeeding or limitation throughout cultivation.

Osmolality

Osmolality remained below 370 mOsmol/kg in all cultures until Day 7, but increased to values between 460 and 563 mOsmol/kg by Day 14 (Figure 9). Although this increase could suggest overfeeding, neither glucose nor lactate concentrations provided any supporting evidence.

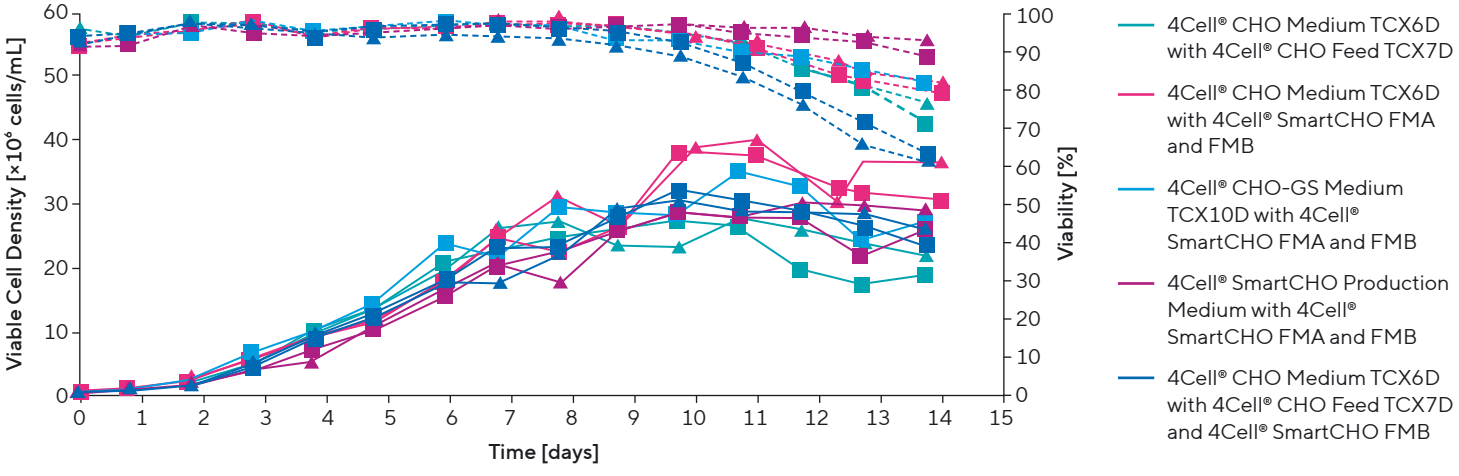
Therefore, residual amino acid concentrations were analyzed in the condition with the highest titer (TCX6D with FMA and FMB) to investigate further.

Amino Acid Concentrations

Amino acid concentrations were analyzed on Days 3, 6, and 12 (Figure 10). Most residual amino acid concentrations remained within 0.5- to 1.3-fold of their concentrations in TCX6D. However, alanine, glutamate, phenylalanine, and tyrosine were present at more than 2-fold higher concentrations compared to the medium, while asparagine was below 0.15 until Day 6.

On average, 52% feed was added to each duplicate. Although reducing the feed rate could decrease osmolality, lowering the feed volume could also increase the risk of nutrient limitations, particularly if consumption rates increase in upscaled processes. Overall, the results indicate that the chosen feed regime avoided excessive overfeeding or nutrient limitations.

Figure 3: *CHO-K1 Viable Cell Density (Solid Lines) and Viability (Dashed Lines) in Ambr® 15 Fed-Batch Cultures With Different Media | Feed Combinations.*



Note. One replicate is displayed as triangles; the other replicate is displayed as squares.

Figure 4: *CHO-K1 Antibody Titers With Different Media | Feed Combinations*

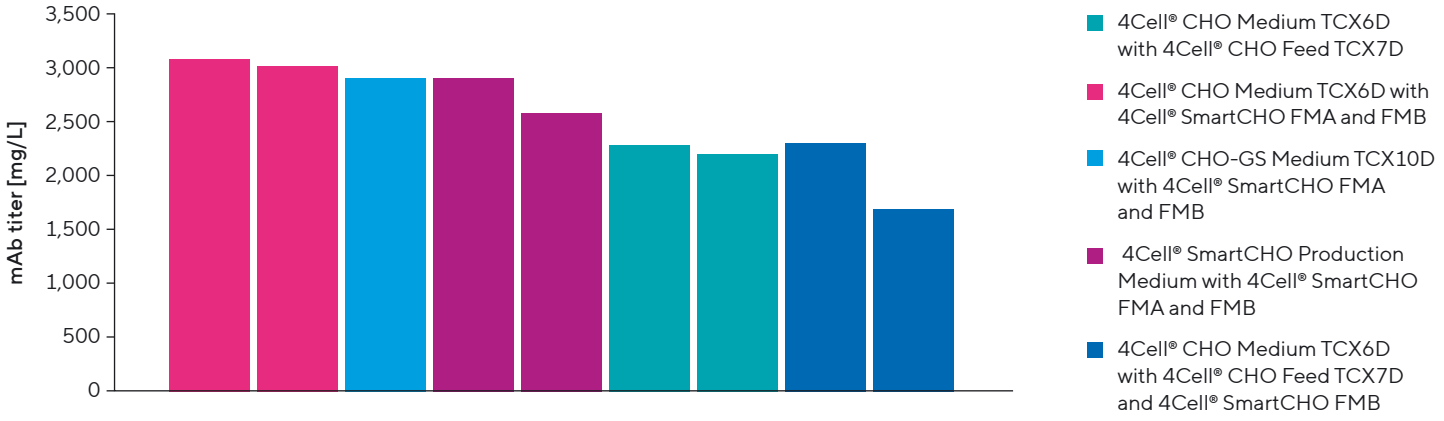


Figure 5: *Product N-Glycosylation Profiles in Final Day Samples of CHO-K1 Ambr® 15 Cultures*

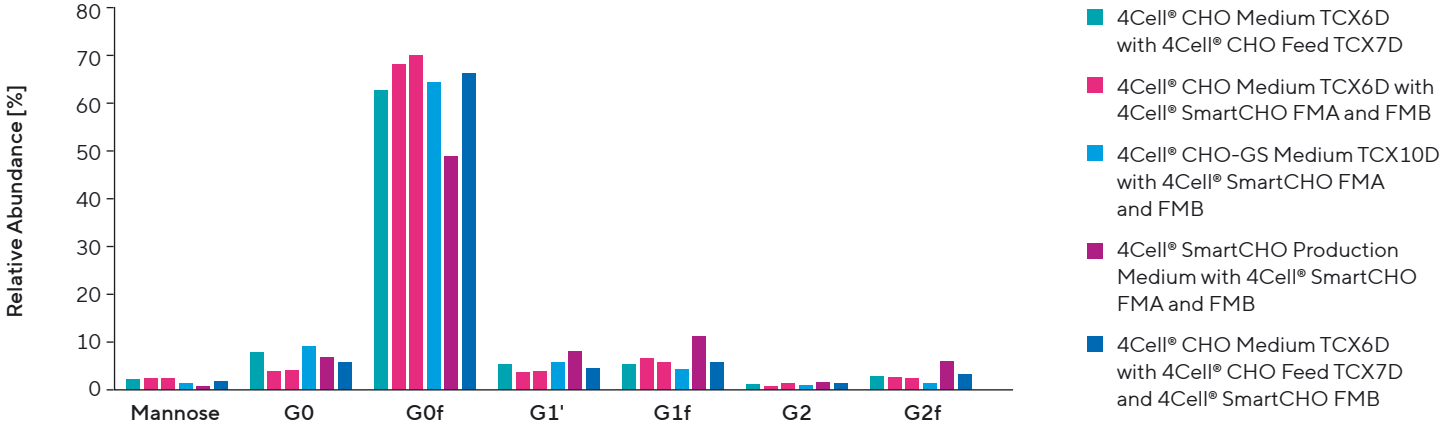


Figure 6: *Product Charge Variant Profiles in Final Day Samples of CHO-K1 Ambr® 15 Cultures*

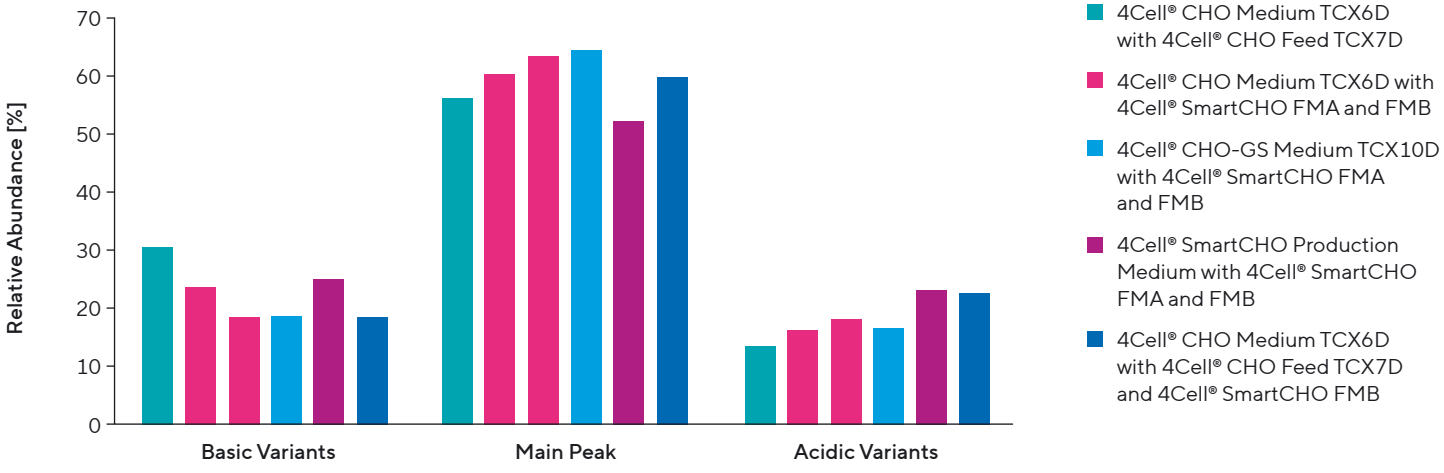
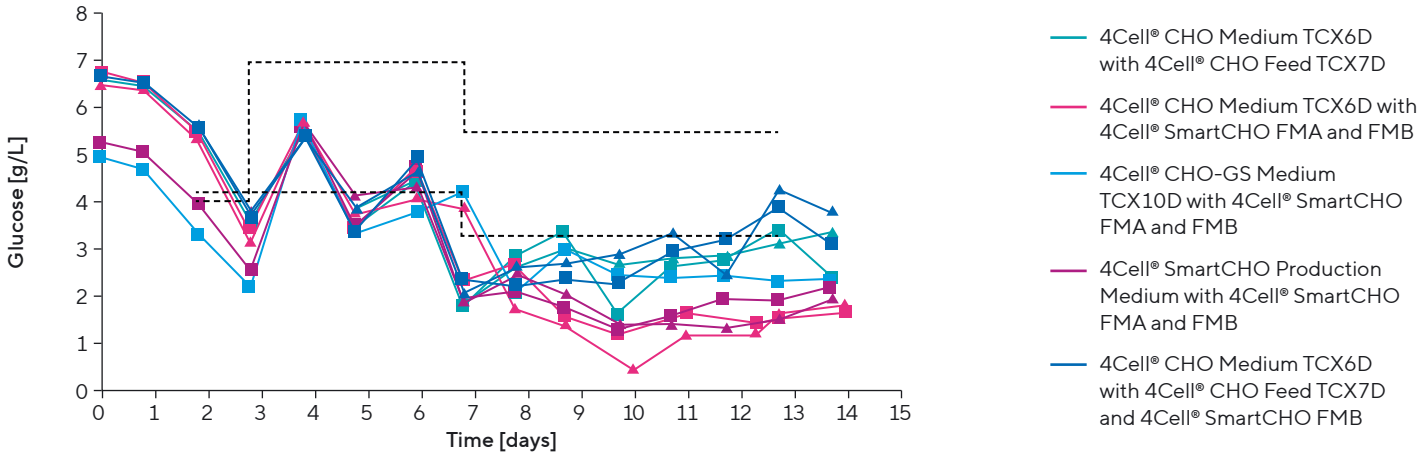
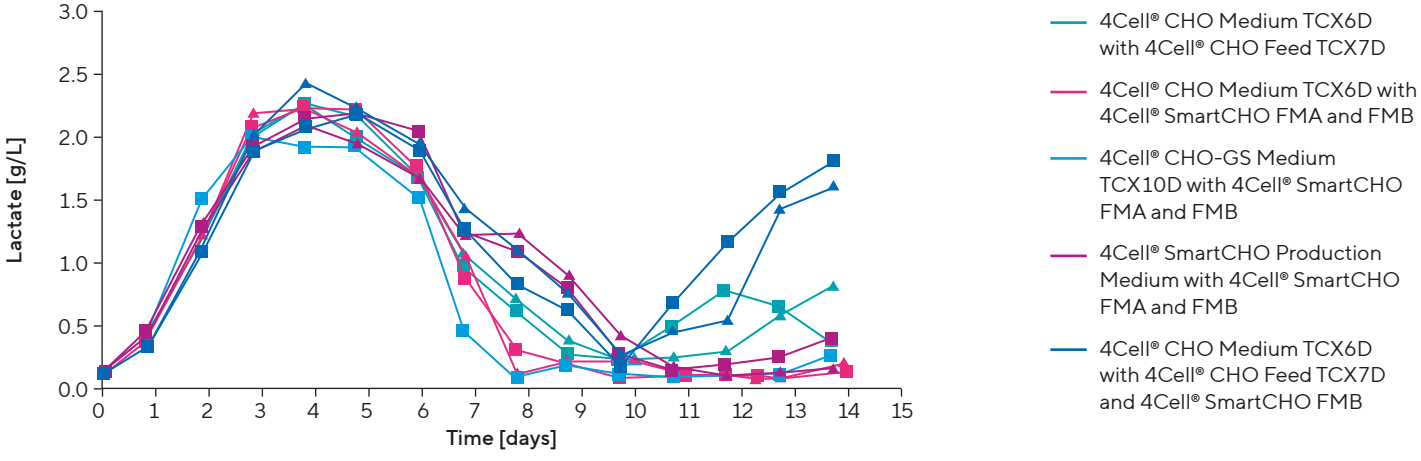


Figure 7: *Residual Glucose Concentrations for CHO-K1 Fed-Batch Cultures in the Ambr® 15 Before Feed Addition*



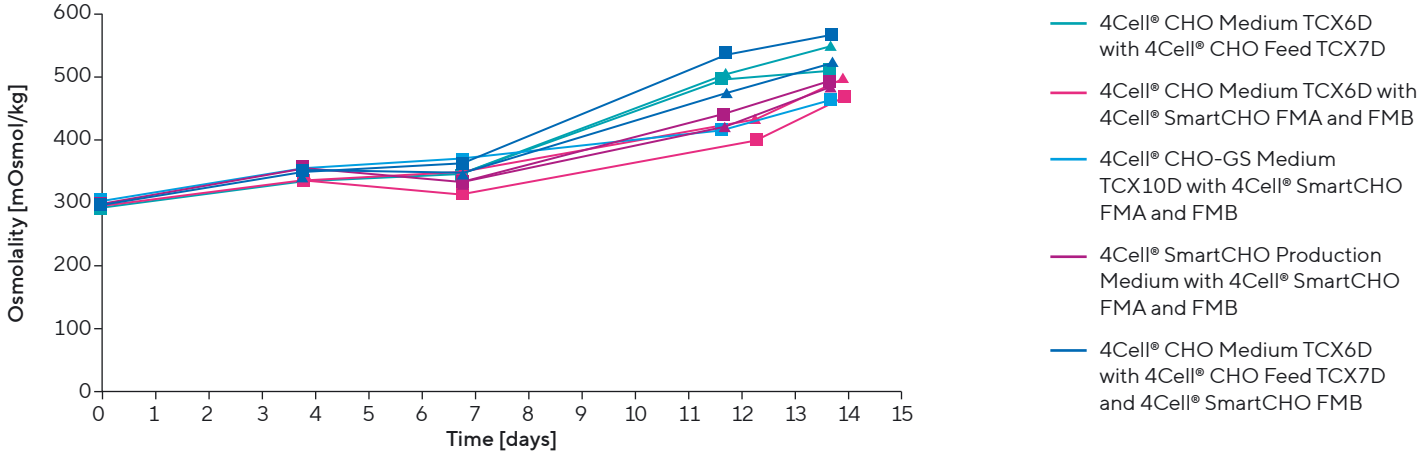
Note. Top dashed line = glucose target concentration, lower dashed line = glucose trigger concentration. One replicate is displayed as triangles; the other replicate is displayed as squares.

Figure 8: *Residual Lactate Concentrations for CHO-K1 Fed-Batch Cultures in the Ambr® 15 Before Feed Addition*



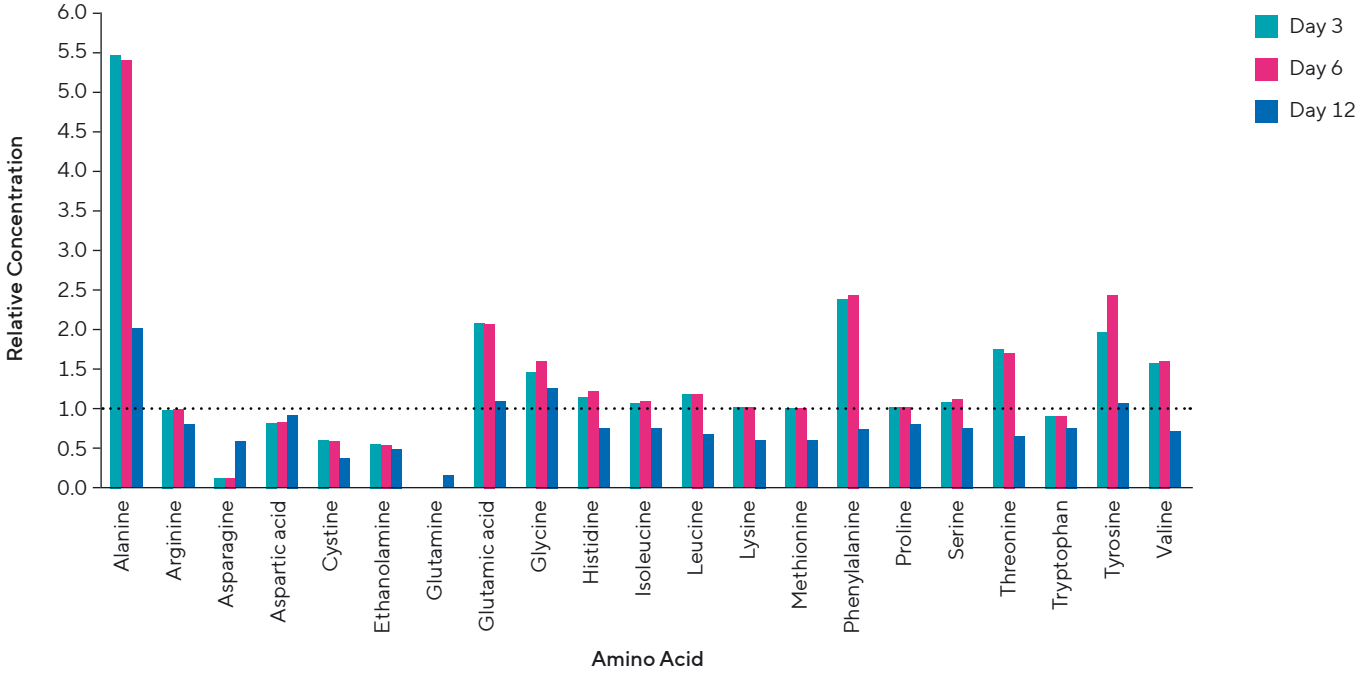
Note. One replicate is displayed as triangles; the other replicate is displayed as squares.

Figure 9: *Osmolality of CHO-K1 Fed-Batch Cultures in Ambr® 15*



Note. One replicate is displayed as triangles; the other replicate is displayed as squares

Figure 10: *Relative Amino Acid Concentrations in 4Cell® CHO Medium TCX6D With 4Cell® SmartCHO FMA and FMB Cultures Compared to Respective Concentrations in the Base Medium*



Note. A value of 1 indicates the same concentration as in the base medium

Ambr® 15 Fed-Batch Cultivation of CHO-S Cells

CHO-S cells were also cultivated in Ambr® 15 fed-batch processes to evaluate different media and feed combinations, again using glucose as the lead substrate for feeding.

Cell Growth and Viability

CHOlean showed the best performance in terms of cell growth and VCD, followed by TCX6D. The lowest growth was observed in SmartCHO (Figure 11). Up to Day 4, cultures in CHOlean and TCX6D exhibited comparable growth. From Day 4 onward, however, CHOlean supported higher cell densities, exceeding 15×10^6 cells/mL by Day 6.

The highest VCDs—19.9×10^6 cells/mL and 20.7×10^6 cells/mL—were achieved using CHOlean with FMA and FMB, corresponding to the highest IVCD of 175×10^6 cells × day/mL. This was followed by cells cultured in CHOlean with TCX7D feed (164×10^6 cells × day/mL) and with TCX7D feed and FMB (155×10^6 cells × day/mL). Slightly lower IVCDs were achieved in TCX6D with TCX7D feed (151×10^6 cells × day/mL) and with FMA and FMB (139×10^6 cells × day/mL). The lowest IVCDs were observed in cultures using SmartCHO: 106×10^6 cells × day/mL with TCX7D feed, and 133×10^6 cells × day/mL with FMA and FMB.

Viability remained above 90% in all cultures until Day 8. In the later phase of cultivation, cultures fed with FMA and FMB maintained higher viabilities than those fed with TCX7D feed, with the exception of cultures using TCX6D with FMA and FMB. The highest overall viabilities were observed in the complete 4Cell® SmartCHO system—SmartCHO PM with FMA and FMB—which showed viabilities between 83% and 91% on Day 14 (Figure 11).

Titer

In contrast to the results with CHO-K1 cells, the choice of base medium had a pronounced impact on titer in CHO-S cultivation (Figure 12). Consistent with the growth performance, the highest titers were observed in cultures using CHOlean, followed by TCX6D, and the lowest in SmartCHO. As observed with CHO-K1 cells, the FMA and FMB feed system outperformed TCX7D feed. The highest overall performance was achieved with CHOlean combined with FMA and FMB, resulting in an average titer of 3,227 mg/L.

No impact was observed by combining FMB with TCX7D feed, as cells cultured in CHOlean with either TCX7D feed or TCX7D feed plus FMB showed comparable titers ranging from 2,648 mg/L to 2,688 mg/L. The combination of TCX6D with FMA and FMB achieved a maximum titer of 2,575 mg/L, which is remarkable considering a temporarily low pH of 6.80–6.83 was recorded for a duration of over four hours.

SmartCHO showed significantly lower titers, with FMA and FMB yielding 2,019 mg/L and TCX7D feed producing only 1,292 mg/L—a 2.5-fold reduction compared to the best-performing condition. Therefore, the combination of CHOlean with FMA and FMB was selected for scale-up. All tested conditions yielded a high monomer content of over 95%.

Glycosylation

As observed in CHO-K1 cultures, G0f was the predominant fraction in CHO-S cultures, comprising 56-68% of the total glycan content. The mannose content was moderate to high, with levels between 6 - 13%. However, in the combination of SmartCHO with TCX7D feed, the mannose content decreased to 3.5%. This combination also exhibited the lowest G0f proportion at 56% and the highest G1' fraction at 8.7%. Aside from this exception, no significant differences in glycosylation profiles were observed among the various media and feed combinations (Figure 13).

Charge Variants

In contrast to the glycan structure, the choice of feed has a pronounced effect on charge variants in CHO-S cultures (Figure 14). Cultures fed with FMA and FMB exhibited a higher proportion of basic variants, ranging from 40% to 46%, compared to those fed with TCX7D feed, which ranged from 31% to 35%. In all cultures, the proportion of acidic variants remained consistently lower than both the main peak (40 - 49%) and the basic variants. The use of TCX7D feed resulted in a minimal increase in the proportion of acidic variants.

Glucose and Lactate Concentrations

The glucose-based feed strategy was also used for the cultivation of CHO-S cells. Cultures fed with TCX7D feed typically remained above the trigger concentration one day after feeding, even after reducing the target and trigger concentrations to 5.0 g/L and 3.0 g/L, respectively, on Day 9. In contrast, cultures fed with FMA and FMB were fed daily after adjusting the target and trigger concentrations (Figure 15).

Lactate levels remained low in most cultures, not exceeding 2.0 g/L, with concentrations decreasing after Days 4 or 5. However, in cultures using TCX6D with FMA and FMB, lactate levels increased steadily from Days 7 and 8—corresponding to the time of decreasing viability—resulting in final lactate concentrations of over 7 g/L and 4 g/L (Figure 16).

Interestingly, lactate levels in SmartCHO with FMA and FMB also rose toward the end of the cultivation. This increase correlated with the higher glucose demand of the culture and lower glucose concentrations during the last two days, despite no strong changes in cell density and viability. As in CHO-K1 cultures, the chosen feed strategy effectively avoided overfeeding and glucose limitation, resulting in overall low lactate accumulation.

Osmolality

Osmolality increased during cultivation, with higher values observed in cultures fed with TCX7D feed on Day 7, ranging from 394 to 432 mOsmol/kg, except for one culture using TCX6D with TCX7D feed. By the end of cultivation, all cultures showed high osmolality, ranging from 468 to 616 mOsmol/kg (Figure 17). These rising values suggest potential overfeeding. However, as glucose and lactate concentrations did not indicate overfeeding, the residual amino acid concentrations were examined in the culture with the highest titer.

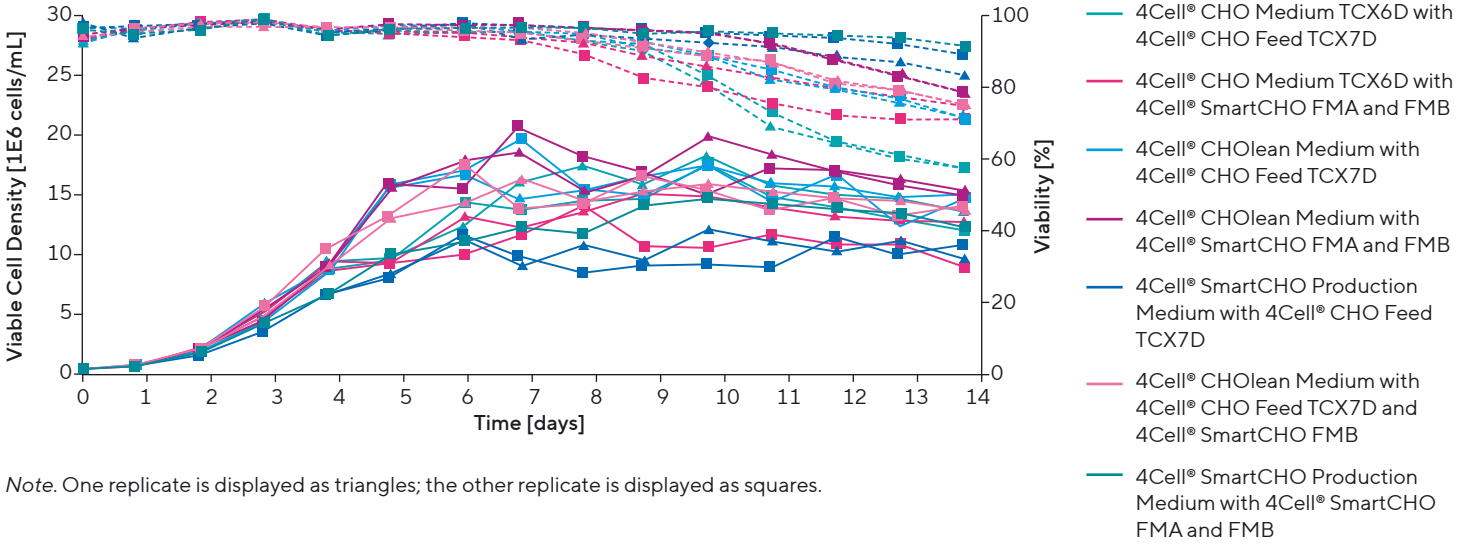
Amino Acid Concentrations

Amino acid concentrations in the CHOlean with FMA and FMB culture were analyzed on Days 3, 6, and 12 (Figure 18). On Days 3 and 6, most amino acids were within 0.5 - 1.2 times the concentrations in of the base medium. However, aspartate, glutamate, glycine, phenylalanine, and tyrosine were 1.8 - 3.9 times higher on Day 6, while asparagine and ethanolamine were only 0.1 and 0.2 times the original concentration in the base medium. By Day 12, most amino acids showed relative concentrations 1.5 - 2.4 times higher than the base medium, with aspartate, glutamate, glycine, phenylalanine, tyrosine, and valine rising 2.8 - 5.8 times. A total of 50% feed was added relative to the inoculation volume. The elevated amino acid concentrations on Day 12 suggest overfeeding during the second half of the cultivation, which also resulted in increased osmolality.

Reducing the feed rate in the second half of the cultivation could lower osmolality. However, the absence of amino acid data between Days 6 and 12 made it difficult to determine when potential overfeeding began. Reducing the feed volume too early could risk limitations, especially if nutrient demand increases in an upscaled process.

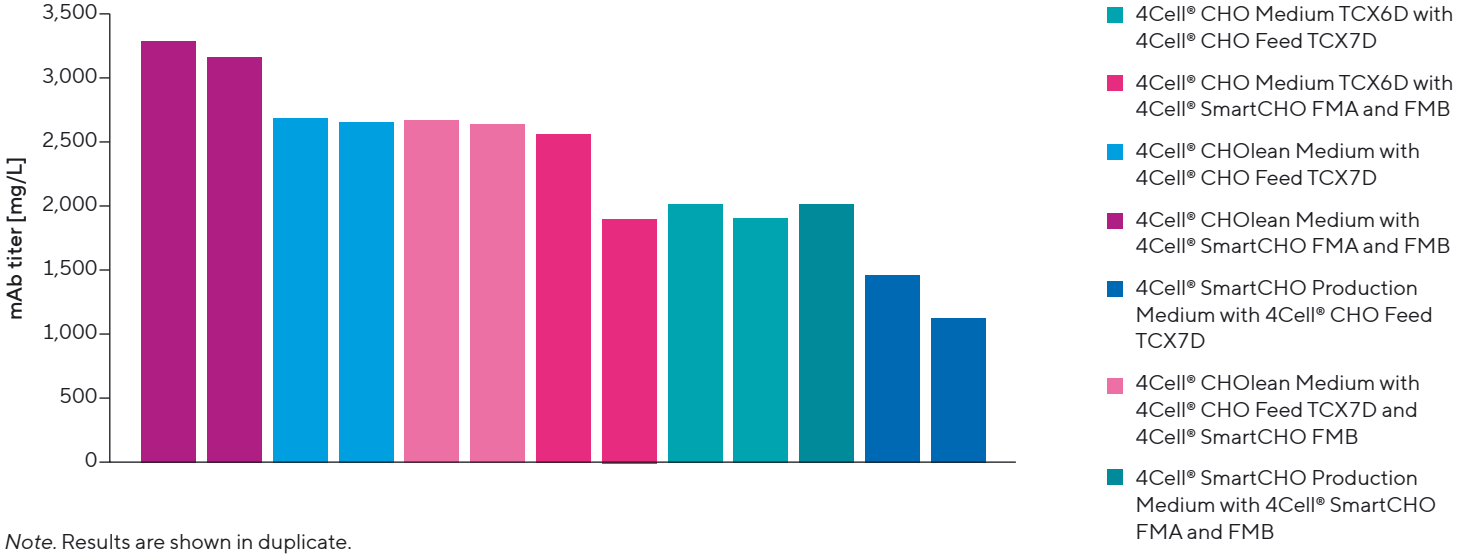
To investigate the lactate increase observed in the TCX6D with FMA and FMB cultures on Day 6, the amino acid profiles of both replicates were analyzed. Based on this analysis, the cell-specific amino acid consumption rates were calculated and compared to those of the CHOlean with FMA and FMB culture. Notably, the culture with the most pronounced lactate production demonstrated substantial net production of aspartate at 0.035 pmol/(cell × day) and glutamate at 0.012 pmol/(cell × day). In contrast, the duplicate culture with delayed lactate production showed lower aspartate production at 0.012 pmol/(cell × day) and net glutamate consumption at -0.009 pmol/(cell × day). Conversely, in the CHOlean with FMA and FMB culture—where lactate consumption occurred—there was no significant net production of aspartate (-0.002 pmol/(cell × day)) and a net glutamate consumption of -0.021 pmol/(cell × day).

Figure 11: *CHO-S Viable Cell Density (Solid Lines) and Viability (Dashed Lines) in Ambr® 15 Fed-Batch Cultures With Different Media | Feed Combinations.*



Note. One replicate is displayed as triangles; the other replicate is displayed as squares.

Figure 12: *CHO-S Antibody Titers With Different Media | Feed Combinations*



Note. Results are shown in duplicate.

Figure 13: *Product N-glycosylation Profiles in Final Day Samples of CHO-S Ambr® 15 Cultures*

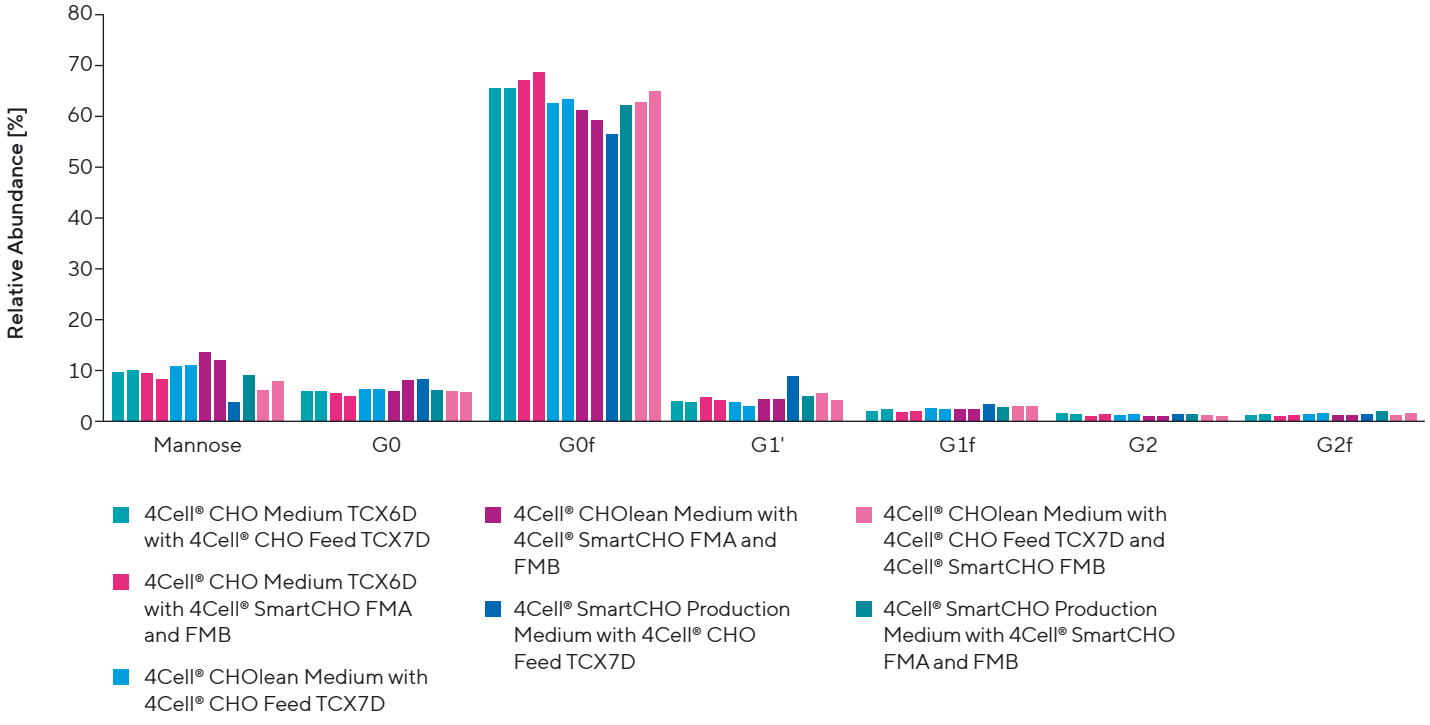


Figure 14: *Product Charge Variant Profiles in Final Day Samples From CHO-S Ambr® 15 Cultures*

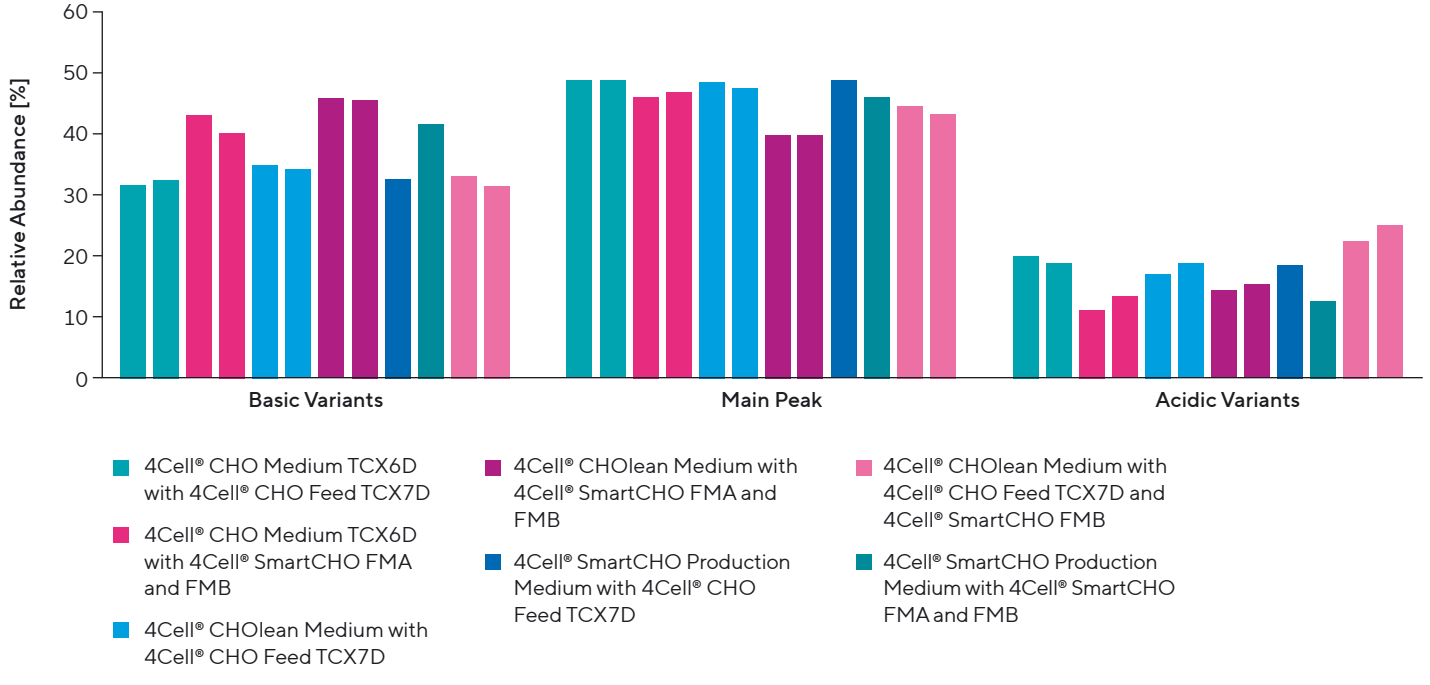
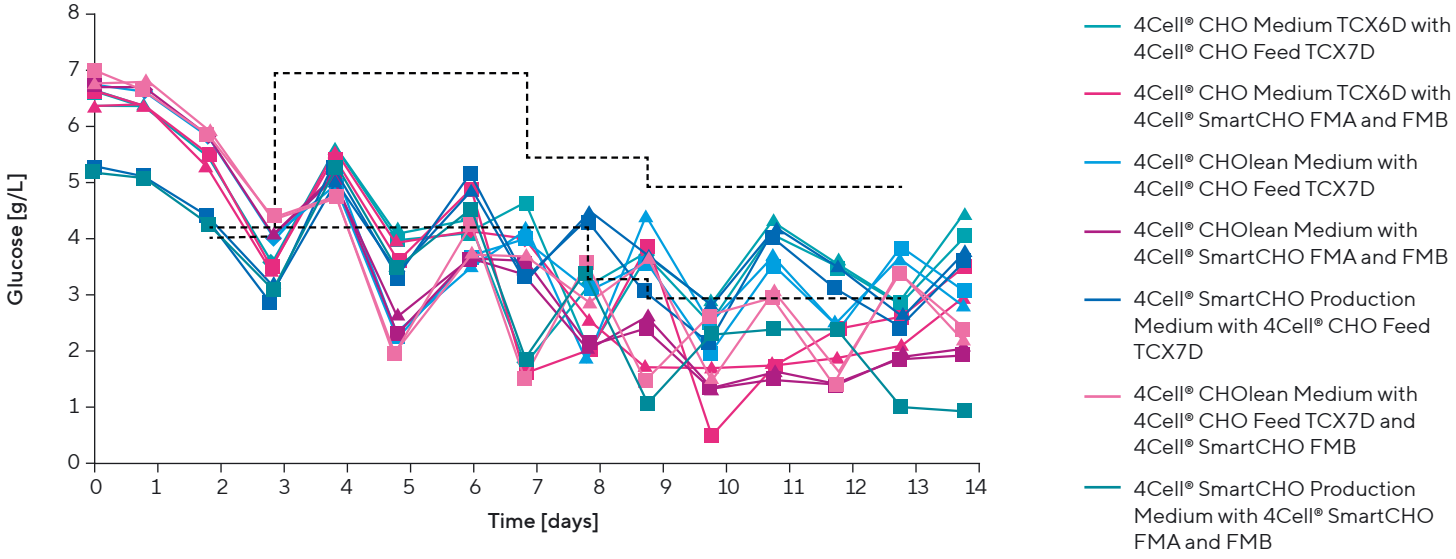
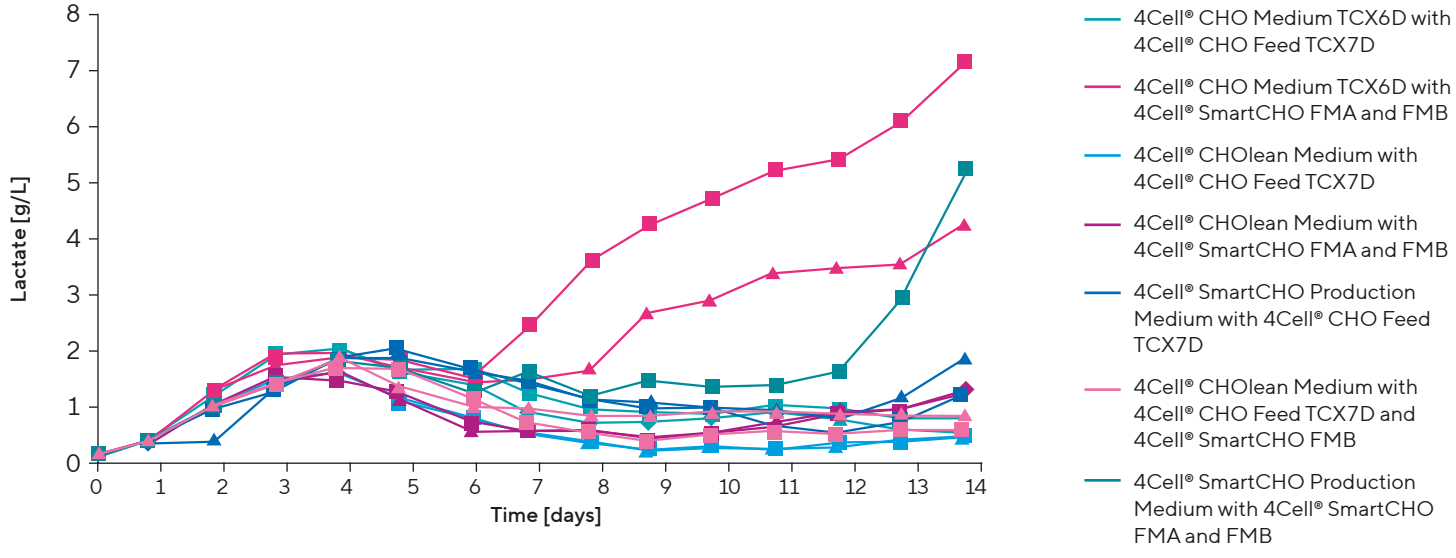


Figure 15: *Residual Glucose Concentrations for CHO-S Fed-Batch Cultures in the Ambr® 15 Before Feed Addition*



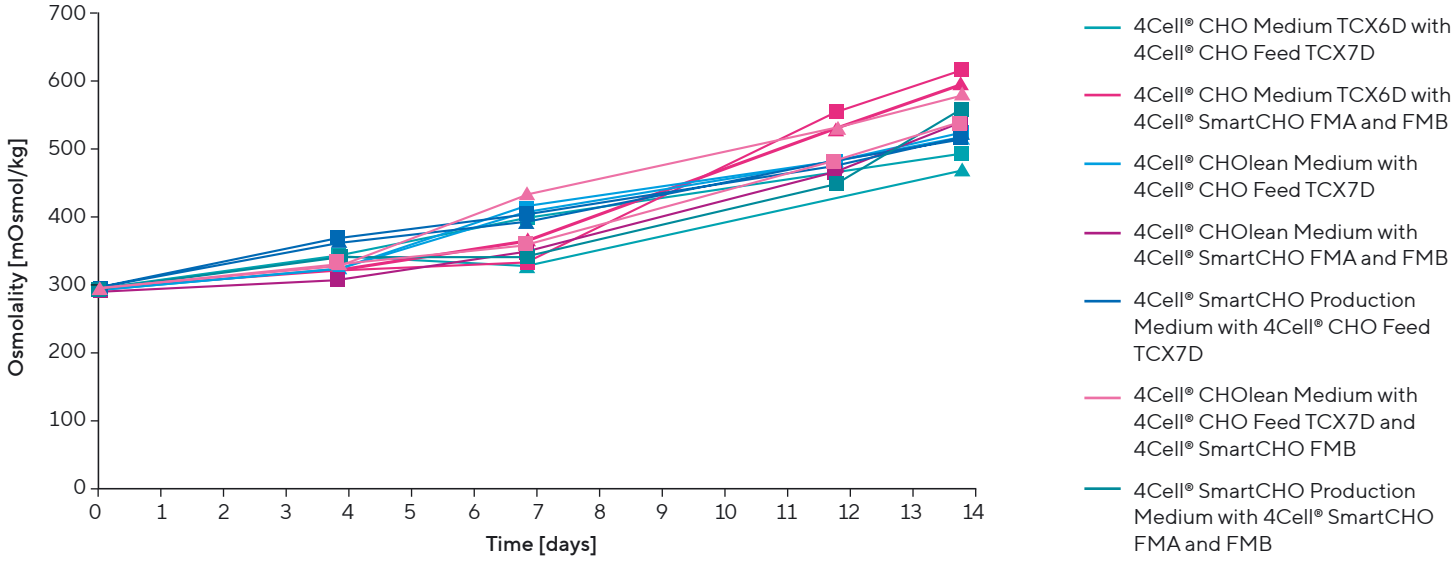
Note. Top dashed line = glucose target concentration, lower dashed line = glucose trigger concentration. One replicate is displayed as triangles; the other replicate is displayed as squares.

Figure 16: *Residual Lactate Concentrations for CHO-S Fed-Batch Cultures in the Ambr® 15 Before Feed Addition*



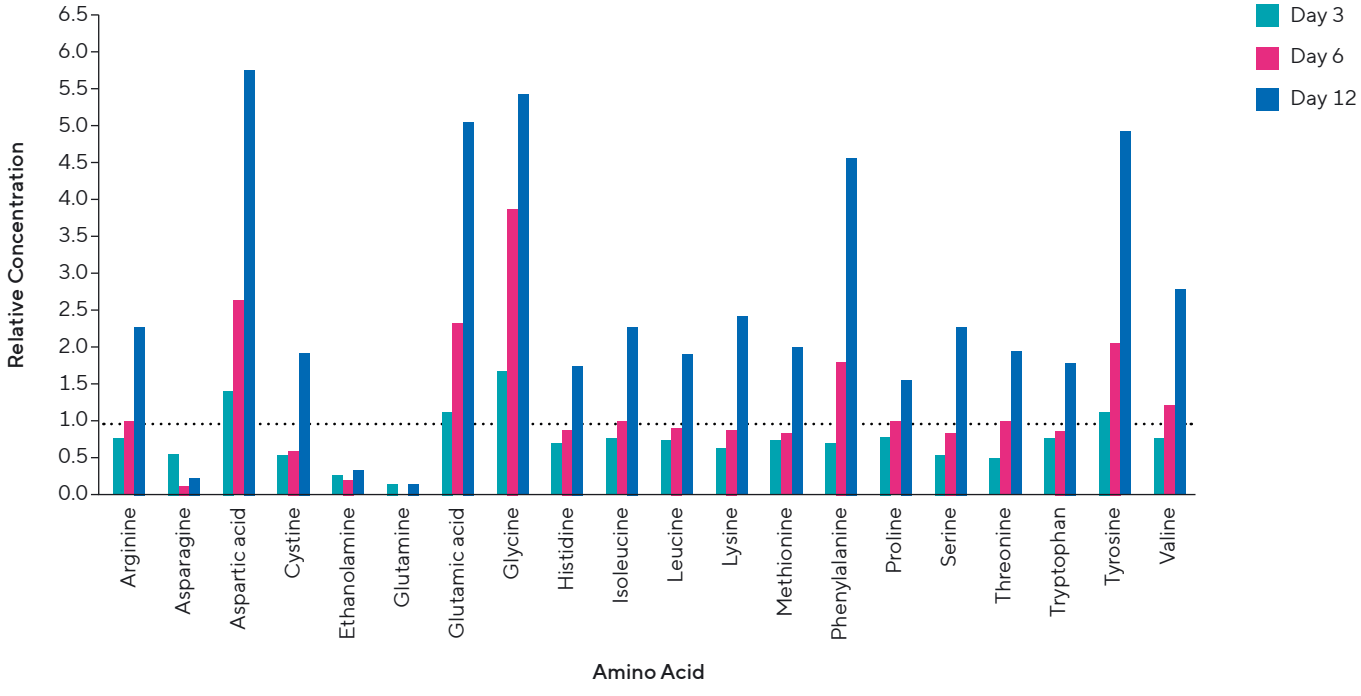
Note. One replicate is displayed as triangles; the other replicate is displayed as squares.

Figure 17: *Osmolality of CHO-S Fed-Batch Cultures in Ambr® 15*



Note. One replicate is displayed as triangles; the other replicate is displayed as squares.

Figure 18: *Relative Amino Acid Concentrations in 4Cell® CHOlean Medium With 4Cell® SmartCHO FMA and FMB Compared to Respective Concentrations in the Base Medium*



Note. A value of 1 indicates the same concentration as in the base medium

2 L Benchtop Bioreactor Cultivation of CHO-K1 and CHO-S Cells

Following the results from the Ambr® 15 fed-batch experiments, the top medium and feed combination for each cell line was scaled up in a 2 L Biostat® B benchtop bioreactor system.

Cell Growth and Viability

Both cell lines demonstrated high growth rates, with maximum growth rates of up to 0.99 d^{-1} (CHO-K1) and 1.08 d^{-1} (CHO-S). CHO-K1 cells reached a peak VCD of 30.5 × 10^6 cells/mL, lower than in the Ambr® 15 run and corresponding to an IVCD of 232 × 10^6 cells × day/mL (Figure 19). In contrast, CHO-S cells achieved a higher peak VCD than in the Ambr® 15 run, reaching 24.5 × 10^6 cells/mL, and an IVCD of 202 × 10^6 cells × day/mL (Figure 20). It is important to note that the analysis was conducted using a Cedex® Cell Counter as opposed to the integrated Vi-CELL® XR used in the Ambr® 15 system. The measurements from the bioreactors were more reliable due to the presence of fewer small particles in the culture.

After reaching peak VCD, cell densities decreased on Day 10 in CHO-K1 cultures and Day 7 in CHO-S cultures. The decline in CHO-K1 cell density correlated with decreasing viability, although the dilution effect from feed addition must be considered. In contrast, the decrease in CHO-S cell density can be attributed to dilution from feed addition, as the total cell number in the bioreactor remained constant until Day 13 (data not shown). Viability was maintained above 96% in both cultures until Day 10. CHO-S cells remained above 88% viability until the end of cultivation, while CHO-K1 cells showed a viability of 75% at the end of cultivation. Therefore, the established process was able to maintain a viability sufficient for industrial production over 14 days.

Glucose and Lactate Concentration

The feed regime for both cell lines in the 2 L bioreactor was based on the strategy established in the Ambr® 15 regime. Using glucose demand calculated from the Ambr® 15 experiments, the feed regime was adjusted to maintain a more stable glucose concentration throughout cultivation. Amino acid analysis results from the Ambr® 15 experiments were also considered.

In the CHO-K1 Ambr® 15 culture, most amino acids did not exceed 1.5-fold of the relative concentration of the TCX6D, and by the end of the cultivation, most relative amino acid concentrations were below those of the base medium (Figure 10). Therefore, glucose was again used as the lead substrate to guide daily feed adjustments in the scaled-up experiment. Although Ambr® 15 CHO-S cultures showed elevated amino acid concentrations by the end of cultivation (Figure 18), the feed volume in the 2 L bioreactor was also based on the glucose demand of the culture. This approach was chosen to avoid nutrient limitations between Days 6 and 12, as described previously, and to enable direct comparison between the Ambr® 15 and 2 L processes.

This strategy proved effective in both cultivations for maintaining appropriate glucose levels. In CHO-K1 cultures, residual glucose concentrations stabilized between 1-2.2 g/L after Day 5 (Figure 21). In CHO-S cultures, glucose was depleted once on Day 7 due to a higher peak VCD in the bioreactor compared with the Ambr® 15. However, from Day 8 onward, the glucose level was efficiently maintained between 0.75 and 2 g/L (Figure 22). To compensate for the depletion event in CHO-S cultures on Day 7, an additional bolus feed of 1.8% FMA and 0.18% FMB was added.

Lactate levels remained below critical concentrations in both processes, peaking at 2.3 g/L in CHO-K1 (Figure 21) and 2.0 g/L in CHO-S (Figure 22) cultures, consistent with the Ambr® 15 results. Lactate concentrations decreased and remained below 0.2 g/L in CHO-K1 and below 0.6 g/L in CHO-S. Notably, 0.4 g/L lactate was still available as an alternative energy source in the CHO-S culture when glucose was depleted on Day 7.

Ammonium Concentration

Ammonium concentrations changed over time in the CHO-K1 culture (Figure 23). Levels increased until Day 4, reaching 3.7 mM, then decreased to 1.5 mM by Day 7, followed by a rise to 5.2 mM on Day 10, and a subsequent decline. The highest concentration on Day 10 correlates with the maximum peak VCD and the onset of decreasing viability. In contrast, the CHO-S culture showed a different trend: ammonium concentrations also increased up to 4.1 mM on Day 4, remained relatively constant until Day 10, followed by a final decrease to 0.6 mM by the end of cultivation (Figure 24). Since cell growth continued as expected until Day 7, ammonium concentrations did not appear to reach toxic levels in the CHO-S culture.

Osmolality

Osmolality profiles in the bioreactors showed good agreement with the Ambr® 15 results, although more feed was added to the bioreactor cultures. Relative to the inoculation volume, a total feed volume of 77% (CHO-S) and 61% (CHO-K1) was added to the cultures. Nevertheless, the CHO-K1 culture showed 317 mOsmol/kg in the bioreactor compared to an average osmolality of 329 mOsmol/kg in Ambr® 15 on Day 7, and 403 mOsmol/kg and 413 mOsmol/kg on Day 12. The final osmolality was lower in the bioreactor run (436 mOsmol/kg, Figure 25) than in the Ambr® 15 run (478 mOsmol/kg, Figure 9).

For CHO-S, osmolality was 351 mOsmol/kg in the Ambr® 15 and 363 mOsmol/kg in the bioreactor on Day 8. The final values were 539 mOsmol/kg in the Ambr® 15 (Figure 17) and 510 mOsmol/kg in the bioreactor (Figure 26) on Day 14. Thus, the larger feed volume did not result in higher osmolality in the bioreactor compared with the Ambr® 15 cultures. However, the increasing osmolality in both cultures during the second half of the cultivation indicated that excessive feed was introduced.

Amino Acid Concentrations

For a more detailed assessment, amino acid concentrations were analyzed for both cultures on Days 3, 7, 10, 12, and 14, with an additional CHO-S sample taken on Day 6 to assess the impact of glucose depletion on Day 7. Results are shown as relative abundance compared with the concentrations in the production medium.

In the CHO-K1 culture, most amino acid concentrations remained below 1.5 times the production medium concentration until cell growth ended on Day 10. Glycine, phenylalanine, threonine, tyrosine, and valine exhibited higher concentrations, up to a 4-fold increase. After Day 10, 13 amino acids exceeded 1.5 times the production medium concentration, with glycine, phenylalanine, threonine, tyrosine, and valine surpassing 3-fold. This increase corresponded with rising glucose levels between Days 10 and 12 and increasing osmolality. Thus, in contrast to the Ambr® 15 cultivation, overfeeding of nutrients in the bioreactor culture after Day 10 was likely. In addition, the timing of increasing amino acid concentrations correlated with declining viability and cell density. No further glucose increase from Day 12 to 14 corresponded with stable amino acid concentrations during this period. Only asparagine, aspartate, ethanolamine, glutamate, and glutamine remained below the production medium concentration on Days 12 and 14 (Figure 27).

For CHO-S, most amino acid concentrations ranged from 0.3-1.0 times the production medium concentration until cell growth stopped on Day 7. The relative concentrations of asparagine and ethanolamine were lower, while aspartate, glutamine, glycine, tyrosine, and phenylalanine were more than 1.5 times higher. From Day 10 onward, relative concentrations increased dramatically, reaching nearly 2-fold or higher compared with the production medium, with aspartate, glutamate, glycine, phenylalanine, and tyrosine increasing more than 5.5-fold. As with CHO-K1, this increase correlated with rising glucose levels and increasing osmolality after Day 7, except for asparagine, ethanolamine and glutamine, which did not increase significantly (Figure 28). Furthermore, the low asparagine and ethanolamine concentrations on Days 6 and 7 correlated with low glucose concentrations and the cessation of cell growth.

Trace Elements

In addition to amino acid analysis, the measurement of trace elements offers valuable insights, as they play a significant role in various cellular functions and metabolic processes, and can significantly impact PQAs. Supernatant samples from CHO-K1 and CHO-S bioreactor cultures were analyzed on Days 3, 7, 10, and 12 (Figures 29-34). Notably, manganese was nearly depleted by Day 7. Copper concentrations also decreased significantly by Day 7. In contrast, zinc concentrations increased after Day 7 compared with Day 3.

Titers

Higher titers were observed in the 2 L Biostat® B bioreactor system compared with the Ambr® 15 cultivations for both cell lines. As expected, titers increased throughout the cultivation period. In CHO-K1, the titer in the bioreactor reached 3.86 g/L, representing a 1.3-fold increase compared with the Ambr® 15 cultivation (Figure 35). This increased titer was associated with a 1.5-fold higher process-specific productivity (17 pg/(cell × d)), possibly due to more stable DO levels in the bioreactor compared with the temporary low DO in the Ambr® 15 at high cell densities. The cell-specific productivity of CHO-K1 cultures ranged from 16 to 21 pg/(cell × d) between Day 7 and Day 14 (Figure 35).

CHO-S cells achieved a titer of 4.55 g/L (Figure 36), a 1.4-fold increase compared with Ambr® 15 cultures. This was achieved by a 1.3-fold increase in process-specific productivity (23 pg/(cell × d)) and higher cell density. Cell-specific productivity between Day 7 and Day 14 increased slightly, from 20 to 27 pg/(cell × d) (Figure 36). Monomer content was stably high over time in both cultures, exceeding 96%.

Glycosylation

In this experiment, the glycosylation profiles were analyzed over time, revealing significant changes for both CHO-K1 (Figure 37) and CHO-S cells (Figure 38). The proportion of G0f increased markedly during the cultivation, rising from 30% to 56% in CHO-K1 cultures and from 42% to 58% in CHO-S cultures. The mannose content also rose from 0% to 4.8% in CHO-K1 cultures and from 0.5% to 12.6% in CHO-S cultures. Conversely, the proportion of galactosylated glycans decreased in both cell lines. In CHO-K1 cultures, the fucosylated fractions G1f and G2f decreased by factors of 2.3 and 3.8, respectively. In CHO-S cultures, all galactosylated glycans were reduced by factors ranging from 1.9 to 3.2. These findings highlight the significant influence of the feed regime on glycosylation.

When comparing glycosylation profiles after 14 days between the 2 L bioreactor and the Ambr® 15 system, CHO-K1 cultures at the larger scale exhibited a 14-percentage-point lower G0f proportion, along with slight increases in G1f (3.6 percentage points), and G2f (1.9 percentage points), as well as a 2.7-percentage-point increase in mannose content (Figure 41). In contrast, the glycosylation profiles of the CHO-S cultures remained comparable between scales after 14 days (Figure 42).

Charge Variants

Similar to the changes observed in glycosylation structure, the charge heterogeneity profiles for both CHO-K1 (Figure 39) and CHO-S (Figure 40) cultures changed over the cultivation period. In both cultures, the proportion of basic variants increased, with CHO-K1 showing a rise of 12 percentage points and CHO-S increasing by 8 percentage points.

Concurrently, the proportion of the main peak decreased in both cultures. When comparing the results with those obtained in the Ambr® 15 system, both cultures exhibited a slightly higher proportion of basic variants and a reduced share of the main peak at the 2 L scale (Figures 43 and 44).

Figure 19: *CHO-K1 Viable Cell Density (Solid Line) and Viability (Dashed Line) Cultured in 4Cell® CHO Medium TCX6D With 4Cell® SmartCHO FMA and FMB in a 2 L Biostat® B Bioreactor*

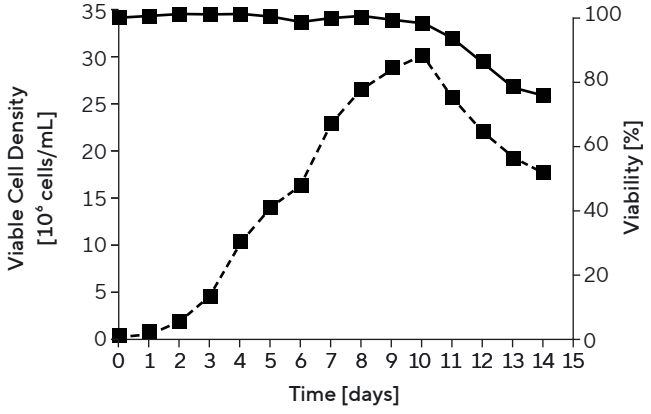


Figure 20: *CHO-S Viable Cell Density (Solid Line) and Viability (Dashed Line) Cultured in 4Cell® CHOlean Medium With 4Cell® SmartCHO FMA and FMB in a 2 L Biostat® B Bioreactor*

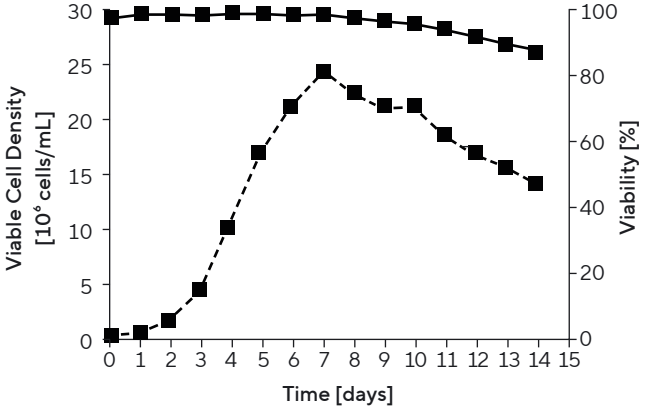


Figure 21: *Residual Glucose (Solid Line) and Lactate (Dashed Line) Concentrations for a CHO-K1 Culture in a 2 L Biostat® B Bioreactor Before Feed Addition*

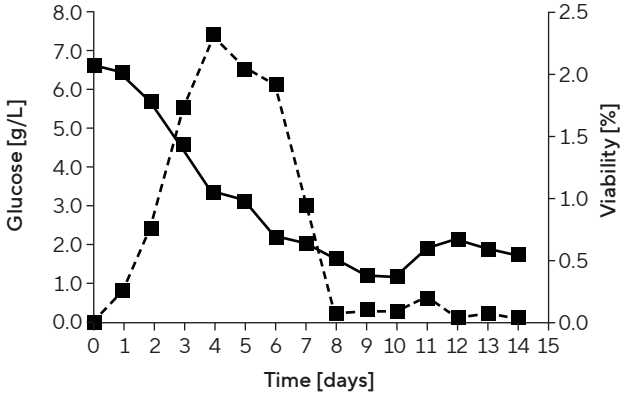


Figure 22: *Residual Glucose (Solid Line) and Lactate (Dashed Line) Concentrations for a CHO-S Culture in a 2 L Biostat® B Bioreactor Before Feed Addition*

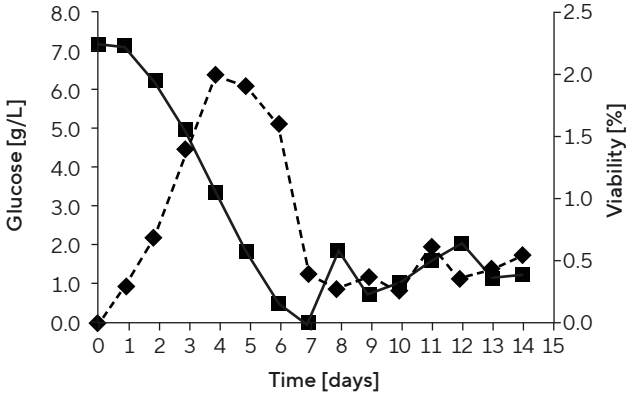


Figure 23: *Ammonium Concentration in a CHO-K1 Culture in a 2 L Biostat® B Bioreactor*

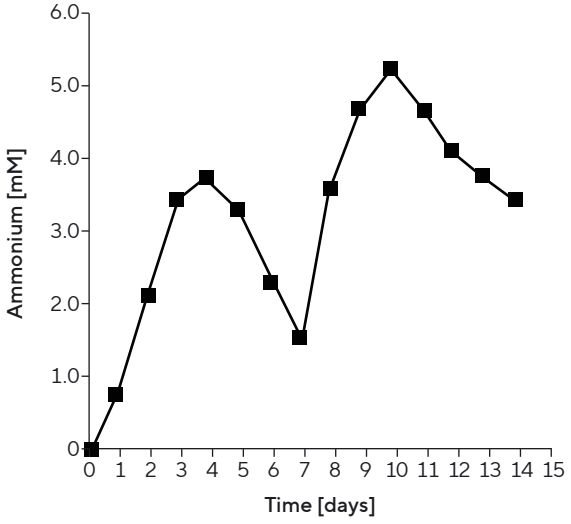


Figure 24: *Ammonium Concentration in a CHO-S Culture in a 2 L Biostat® B Bioreactor*

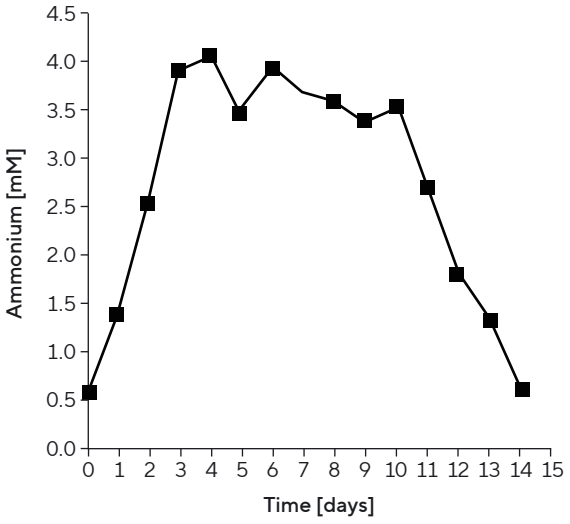


Figure 25: *Osmolality of a CHO-K1 Culture in a 2 L Biostat® B Bioreactor*

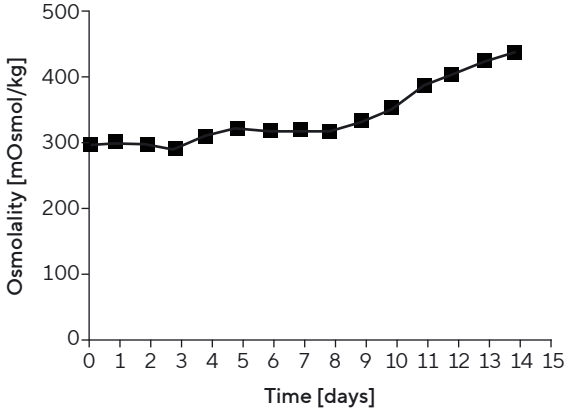


Figure 26: *Osmolality of a CHO-S Culture in a 2 L Biostat® B Bioreactor*

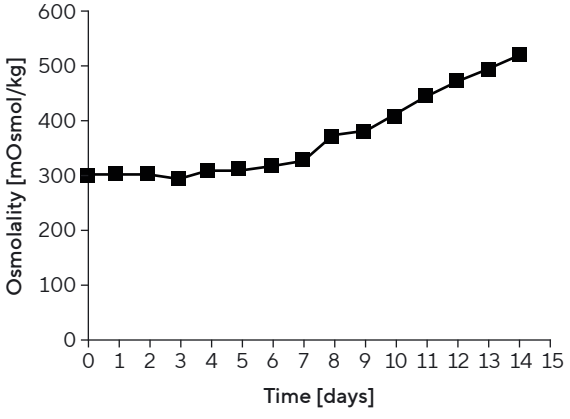
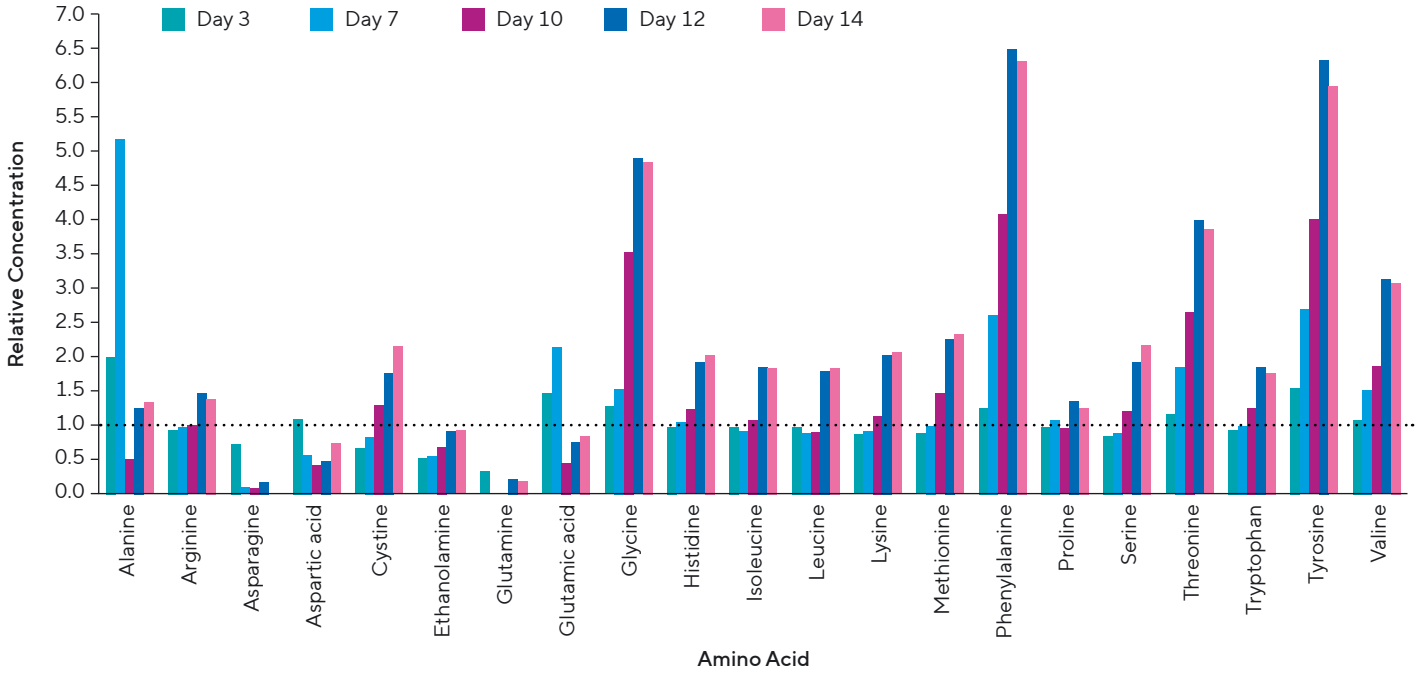
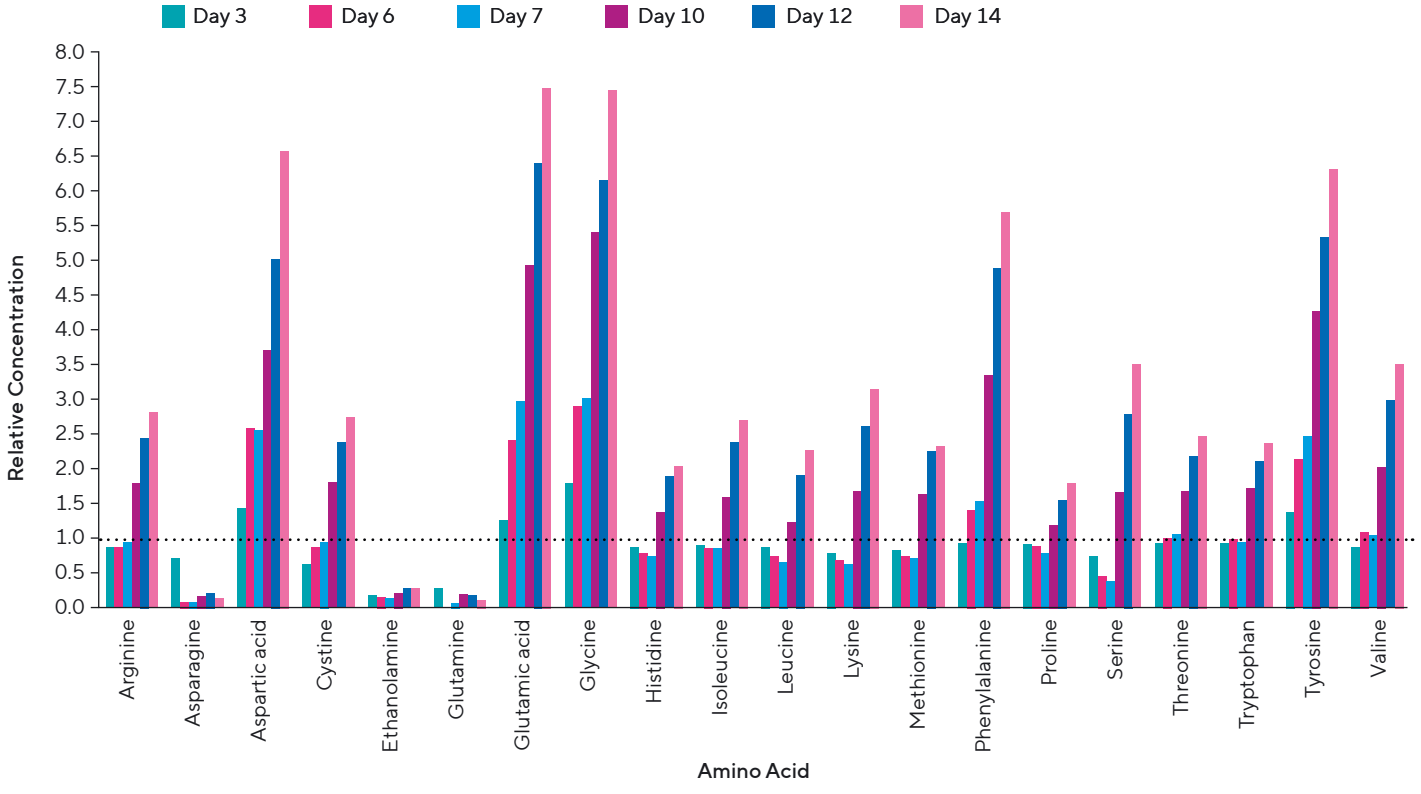


Figure 27: *Relative Amino Acid Concentrations in a 2 L Biostat® B Bioreactor Culture of CHO-K1 Cells Compared With Respective Concentrations in 4Cell® CHO Medium TCX6D*



Note. A value of 1 indicates the same concentration as in the base medium

Figure 28: *Relative Amino Acid Concentrations in a 2 L Biostat® B Bioreactor Culture of CHO-S Cells Compared With Respective Concentrations in 4Cell® CHOlean Medium*



Note. A value of 1 indicates the same concentration as in the base medium

Figure 29: *Relative Manganese (Mn) Concentrations in a CHO-K1 Culture in a 2 L Biostat® B Bioreactor*

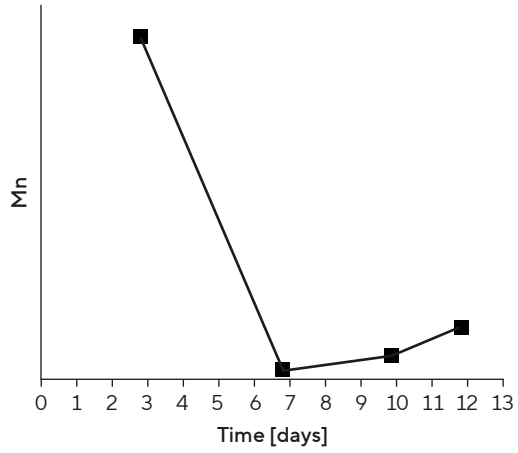


Figure 30: *Relative Manganese (Mn) Concentrations in a CHO-S Culture in a 2 L Biostat® B Bioreactor*

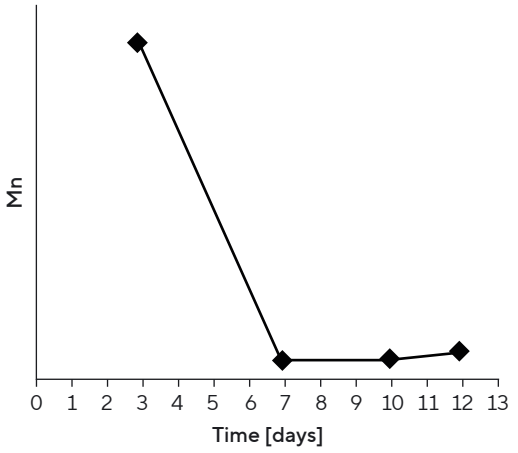


Figure 31: *Relative Copper (Cu) Concentrations in a CHO-K1 Culture in a 2 L Biostat® B Bioreactor*

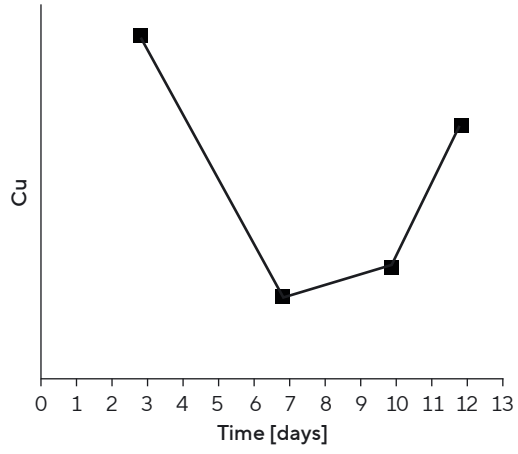


Figure 32: *Relative Copper (Cu) Concentrations in a CHO-S Culture in a 2 L Biostat® B Bioreactor*

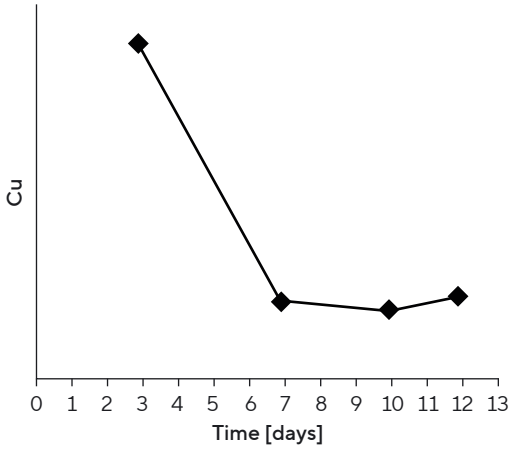


Figure 33: *Relative Zinc (Zn) Concentrations in a CHO-K1 Culture in a 2 L Biostat® B Bioreactor*

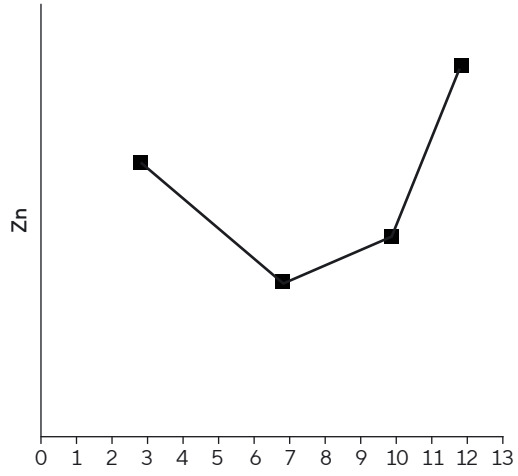


Figure 34: *Relative Zinc (Zn) Concentrations in a CHO-S Culture in a 2 L Biostat® B Bioreactor*

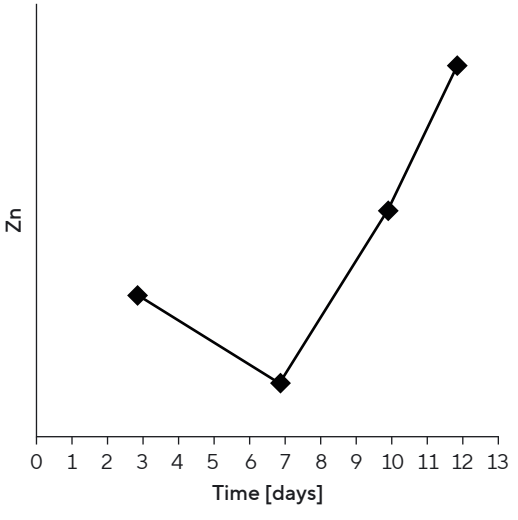


Figure 35: *Antibody Titer (Line) and Cell-Specific Productivity (qP; Diamonds) in a 2 L CHO-K1 Biostat® B Bioreactor Culture*

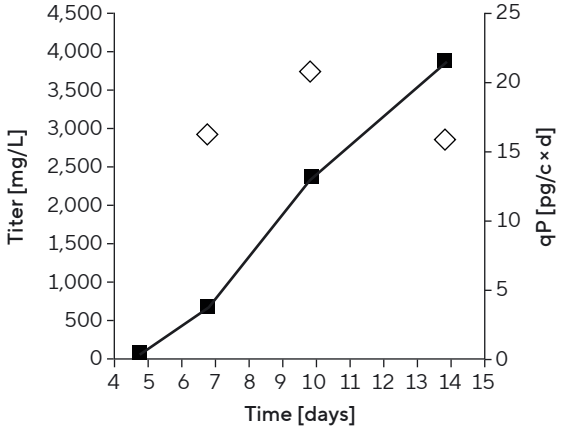


Figure 36: *Antibody Titer (Line) and Cell-Specific Productivity (qP; Diamonds) in a 2 L CHO-S Biostat® B Bioreactor Culture*

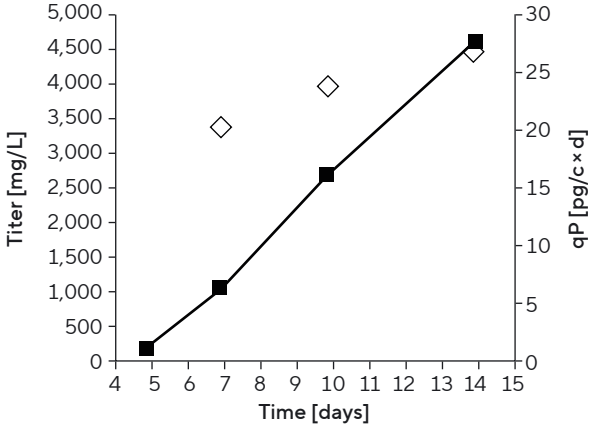


Figure 37: *Product N-Glycosylation Profiles in a CHO-K1 2 L Biostat® B Bioreactor Culture at Different Time Points*

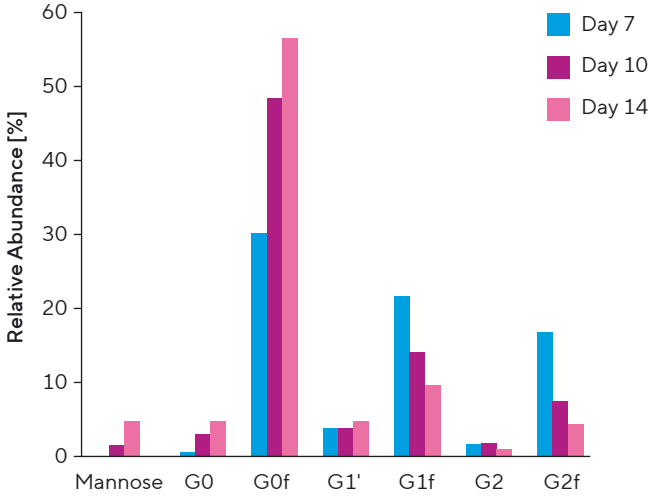


Figure 38: *Product N-Glycosylation Profiles in a CHO-S 2 L Biostat® B Bioreactor Culture at Different Time Points*

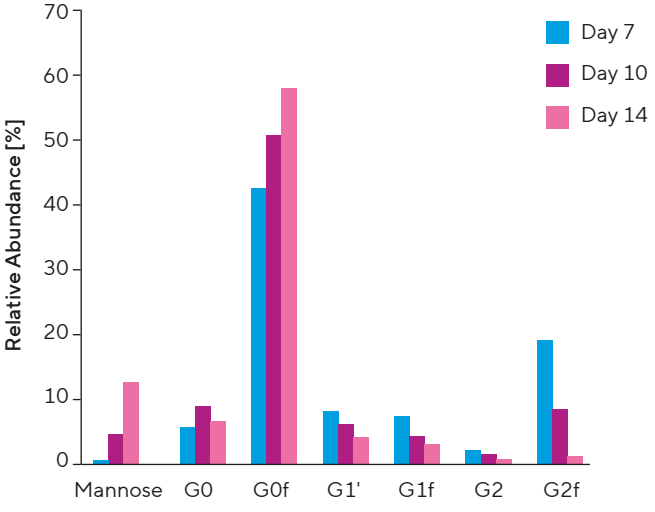


Figure 39: *Charge Variant Profiles in a CHO-K1 2 L Biostat® B Bioreactor Culture at Different Time Points*

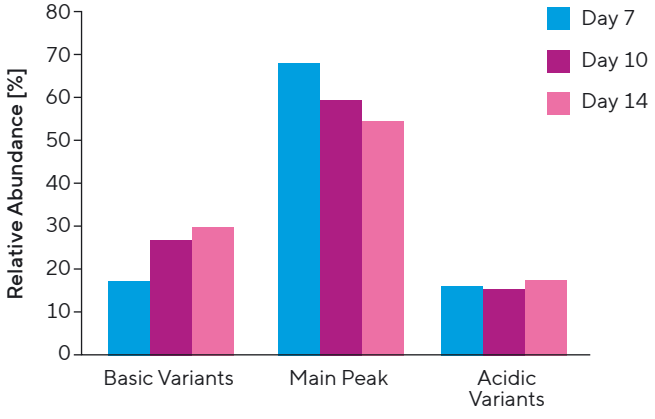


Figure 40: *Charge Variant Profiles in a CHO-S 2 L Biostat® B Bioreactor Culture at Different Time Points*

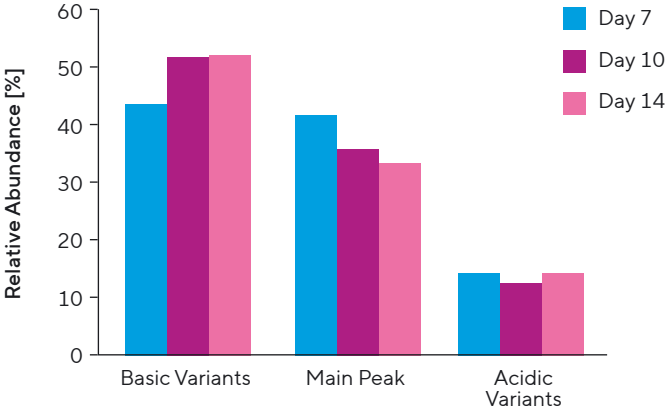


Figure 41: *N-Glycan Profiles of CHO-K1 Cultures in 4Cell® CHO Medium TCX6D With 4Cell® SmartCHO FMA and FMB: Comparison Between 2 L Biostat® B and Duplicate Cultures in Ambr® 15*

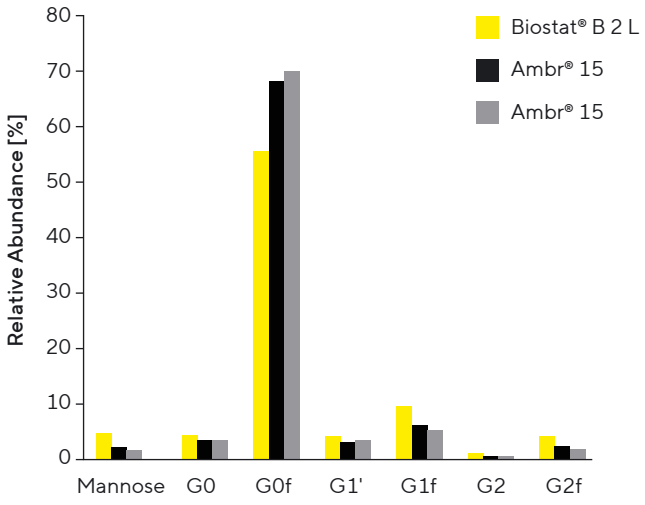


Figure 42: *N-Glycan Profiles of CHO-S Cultures in 4Cell® CHO Medium TCX6D With 4Cell® SmartCHO FMA and FMB: Comparison Between 2 L Biostat® B Bioreactor and Duplicate Cultures in Ambr® 15*

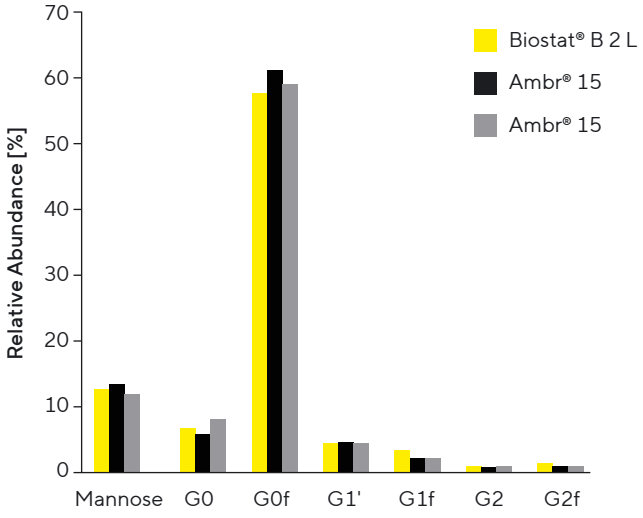


Figure 43: *Charge Heterogeneity in CHO-K1 Cultures in 4Cell® CHO Medium TCX6D With 4Cell® SmartCHO FMA and FMB: Comparison Between 2 L Biostat® B Bioreactor and Duplicate Cultures in Ambr® 15*

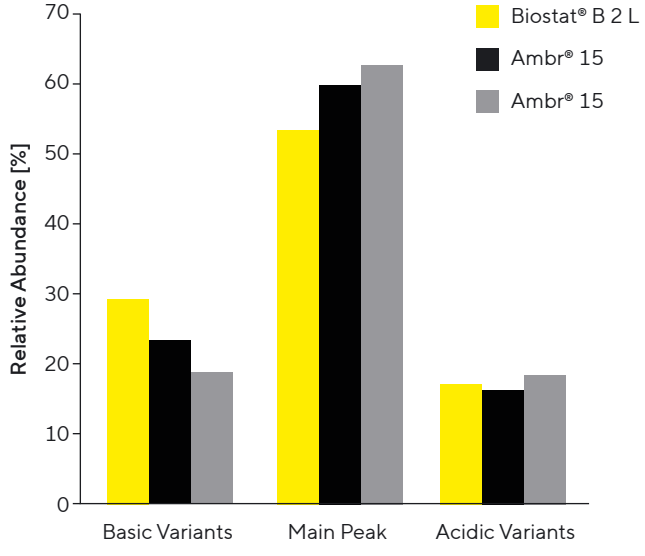


Figure 44: *Charge Heterogeneity in CHO-S Cultures in 4Cell® CHO Medium TCX6D With 4Cell® SmartCHO FMA and FMB: Comparison Between 2 L Biostat® B Bioreactor and Duplicate Cultures in Ambr® 15*

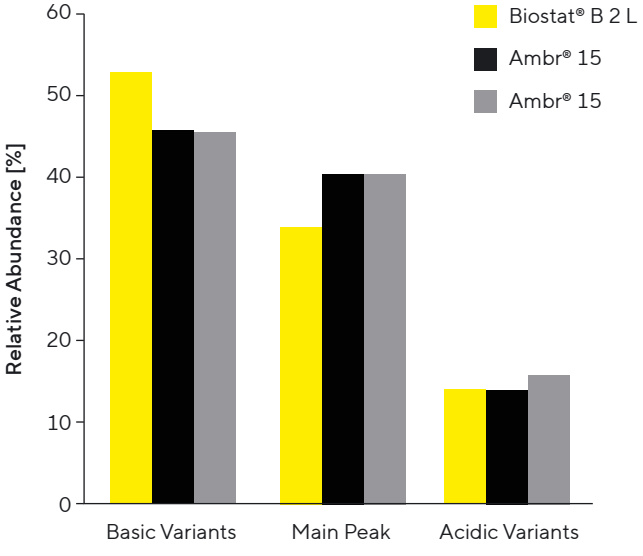


Table 6: *Summary of mAb-Producing CHO-K1 and CHO-S Cell Cultivation in Ambr® 15 and a 2 L Biostat® B Bioreactor.*

Parameter		CHO-K1		CHO-S	
		Ambr® 15	**2 L Biostat® B**	**Ambr® 15**	**2 L Biostat® B**
	Optimal production medium	4Cell® CHO Medium TCX6D		4Cell® CHOlean Medium	
	Optimal feed	4Cell® SmartCHO FMA and FMB		4Cell® SmartCHO FMA and FMB	
	Peak VCD [10^6 cells/mL]	38.1 – 40.3	30.5	19.9 – 20.7	24.5
	IVCD [10^6 cells/mL × day]	283 – 297	232	172 – 177	202
	Total feed volume based on inoculation volume [%]	51 – 53	61	49 – 50	77
	Max. osmolality [mOsmol/kg]	462 – 494	436	536 – 541	510
	mAb titer [g/L]	3.02 – 3.10	3.86	3.17 – 3.28	4.55
	Max. q_{mab} [pg/(cell × day)]	–	20.7	–	26.6
	Process q_{mab} [pg/(cell × day)]	10 – 11	17	18 – 19	23
	mAb monomer content [%]	97.6 – 97.8	97.9	96.5, 96.5	96.4
N-glycan profile at day 14	Mannose [%]	1.9 – 2.2	4.8	11.8 – 13.3	12.6
	G0 [%]	3.7 – 3.8	4.5	5.7 – 7.9	6.6
	G0f [%]	68.7 – 70.5	56.1	58.9 – 61.0	57.7
	G1' [%]	3.6 – 3.7	4.5	4.3 – 4.4	4.3
	G1f [%]	6.5 – 5.5	9.6	2.1, 2.1	3.2
	G2' [%]	0.7 – 0.9	1.2	0.7, 0.7	0.8
	G2f [%]	2.6 – 2.3	4.4	0.9, 0.9	1.2
Charge variants at day 14	Basic variants [%]	19 – 24	29	45 – 46	52
	Main peak [%]	60 – 63	54	40, 40	34
	Acidic variants [%]	16 – 18	17	14 – 15	14

Note. Duplicates are shown separated by "– " or ","

Discussion

Media and Feed Impact

In this study, we explored the impact of various production media and feed combinations on mAb titer and PQAs. Results from experiments in the Ambr® 15 clearly demonstrated that the influence of media and feed on product titer is cell line dependent. For CHO-S cells, both media and feed significantly affected the titer, with the production medium having the most substantial impact. Conversely, the titer of CHO-K1 cells was primarily driven by the choice of feed. The need to screen different media and feed combinations—due to their variable, cell line–specific effects on titer—has also been corroborated by previous studies.[7]

In addition to identifying conditions for maximizing titer, screening different media and feed combinations provided an effective way to assess process robustness. In this study, the combination of TCX6D with FMA and FMB resulted in substantial lactate accumulation in CHO-S, which is undesirable. Using SmartCHO and FMA and FMB maintained a constant lactate level, with an increase observed at the end of cultivation in this cell line. In contrast, all other combinations led to lactate consumption during the second half of cultivation, offering a more robust process.

Spent media analysis and the calculation of consumption rates revealed significant differences in aspartate and glutamate metabolism between CHO-S cultures that accumulated lactate and those that consumed it. Aspartate and glutamate are important amino acids in the TCA cycle and the malate-aspartate shuttle, both of which are key pathways determining the NADH/NAD+ ratio in cells. Imbalances in these pathways can affect cellular energy metabolism and the NADH/NAD^+ ratio, leading to lactate accumulation.[8]

Because the shift from lactate production to consumption is influenced by numerous process parameters,[9] the choice of base medium, feed, and feed regime is critical in determining the metabolic state of the culture.[6] The production of aspartate and, later, glutamate, in lactate-producing cultures during the second half of cultivation could indicate high TCA cycle activity, resulting in net production and accumulation of aspartate and glutamate in the cytosol and, subsequently, in the cultivation medium. High TCA activity produces NADH and can overburden the mitochondrial capacity to regenerate NAD^+, resulting in cytosolic NAD^+ regeneration using cytosolic NADH and, thus, lactate formation.[8]

Another critical factor in selecting a process medium and feed is protein quality. Because media and feed influence product titer, it is essential to screen multiple media and feed combinations to identify the optimal setup. However, if budget constraints limit the scope of screening, focusing on FMA and FMB may be an effective strategy, as our experiments showed that they consistently delivered higher titers across all cell lines, including CHO-GS (as noted in a previous white paper).[6]

While our study confirmed that media and feed had a significant impact on product titer, their effect on the glycosylation profile was much smaller—except for the combination of SmartCHO medium with FMA and FMB with CHO-K1 cells, which resulted in a markedly lower proportion of G0f species. Although minor differences were noted in the Ambr® 15 screening experiments depending on the chosen medium or feed, no substantial changes in the overall glycosylation profile were observed.

However, findings from other studies vary. One study reported a more substantial impact of media and feed on the glycan pattern, suggesting that this influence is highly clone- and product-dependent.[10] Gyorgypal et al. (2025) demonstrated that the impact of the base medium and feed on the glycan profile varies with different media or feeds, depending upon their specific combination.[7] Conversely, Reinhart et al. (2015) did not observe significant differences in glycan profiles across various media and feed combinations.[1]

In our experiments, the feed regime's influence was evident in the 2 L experiments, as indicated by the changes in the glycan profile from Day 7 to Day 14. Therefore, the differences observed between the Ambr® 15 cultures—initially attributed to medium or feed—may also be a consequence of variations in feed regimes resulting from differing metabolic consumption patterns. Furthermore, the combination of SmartCHO with TCX7D feed showed the most pronounced difference in the glycosylation profile of CHO-S cultures, but also the lowest titer. This observation aligns with previous reports suggesting that higher titers can reduce antibody residence time in the Golgi apparatus, thereby limiting glycan processing and reducing galactosylation.[11]

In contrast, a notable impact of feed on charge variant heterogeneity was observed in CHO-S cultures, with cultures fed with FMA and FMB exhibiting a higher proportion of basic variants. Achieving comparable charge variant profiles is crucial to the production of biosimilars, as these variants impact the pharmacokinetics and pharmacodynamics of antibodies.[3]

Therefore, selecting the TCX7D feed may be advantageous when a lower abundance of basic charge variants is required. Similar to the glycan profile, the charge heterogeneity also changed over time in the 2 L bioreactor experiments, underscoring the importance of the feed regime and process conditions in shaping PQAs.

Ambr® 15

The Ambr® 15 system has proven to be an efficient tool for screening various media and feed combinations. Process parameters such as pH and oxygen supply can significantly impact titer, PQAs, and overall culture performance. Without tight control over these variables, additional factors may confound the decision-making process, potentially leading to a suboptimal medium and feed combination. This is exemplified by the differences in total base addition across the Ambr® 15 cultures.

Moreover, without measuring DO, limitations at high cell densities—as observed in CHO-K1 cells cultured in TCX6D with FMA and FMB—would remain undetected. In addition to controlling pH and DO, the Ambr® 15 enables the automation of sampling, feeding, and cell analysis via an integrated cell counter. This automation enhances process control and streamlines the screening of a media and feed panel, increasing the likelihood of identifying the best combination based on performance, without the influence of uncontrolled parameters.

Scale-Up

While the Ambr® 15 proved invaluable for identifying optimal media and feed combinations, the study also highlights the importance of confirming these results in a scaled-up system, such as an Ambr® 250 or a 2 L Biostat® B bioreactor. Higher titers were observed for both cell lines in the larger-scale system. Furthermore, the glucose demand of both cultures was higher in the 2 L bioreactor, and CHO-S cells achieved higher cell densities. The stable oxygen supply in the 2 L bioreactor—unlike the temporary limitations in the Ambr® 15—likely contributed to the higher titer and glucose demand of CHO-K1 cells. As such, the 2 L system provides more reliable data for future large-scale production processes. For example, the pH profile can be maintained within a narrower deadband in the 2 L system, due to the increased volume and the use of more precise pH probes.

The glycan pattern and charge heterogeneity were consistent between the Ambr® 15 and the 2 L system. Although slight deviations were observed, they remained within a range that supports the suitability of using the Ambr® 15 for PQA screening. These findings confirm that the effects of media and feeds on PQAs can be effectively evaluated and, if necessary, optimized during Ambr® 15 screening.

Feed Regime

In this study, the aim was not to develop an optimal feed regime but rather to identify the best combination of media and feed, minimizing the impact of non-optimal feeding so that differences were primarily due to the tested combinations. However, an efficient screening strategy can also provide data for optimizing the feed regime, reducing the effort needed for further process optimization.

The trigger | target feeding strategy used in the Ambr® 15 experiments, with glucose as the lead substrate, simplified the screening process. With just one experiment in duplicate for each combination, this approach immediately provided a feed regime that supported consistent glucose and lactate profiles, avoiding glucose limitations, overfeeding, or high lactate levels, despite varying culture profiles and glucose demand. The "Add Liquid by Concentration" step in the Ambr® 15 program simplified the programming, as described in a previous white paper.[6]

Glucose was also used as the lead substrate for determining the feed regime in the 2 L experiment, combined with the expected glucose consumption data from the Ambr® 15 cultivation. The higher glucose demand of the CHO-S culture led to glucose limitation on Day 7. At this point, lactate was still available as an alternative energy source, and most amino acids showed no limitations—although asparagine and glutamine had low relative concentrations. It remains unclear whether cell growth stopped because the culture reached maximum cell density in combination with the temperature shift or whether the glucose limitation and low concentrations of asparagine and glutamine also contributed to the growth arrest. However, in the Ambr® 15 cultivation of CHO-S cells, low asparagine and glutamine concentrations were also observed in the CHOlean with FMA and FMB around the time of growth arrest—despite sufficient glucose availability. This suggests that low concentrations of these two amino acids may have played a role in halting cell growth. The importance of asparagine in cell proliferation has also been reported previously.[2,12]

To assess the feed regime, it is important to measure the residual concentrations of amino acids in a culture. Overall, the residual amino acid concentrations was well adjusted for most amino acids, in both the CHO-S CHOlean with FMA and FMB and the CHO-K1 TCX6D with FMA and FMB cultures at both scales, as long as the residual glucose concentration decreased. Increasing amino acid concentrations were noted when the glucose concentration stabilized or slightly increased in the bioreactor cultures and the corresponding CHO-S Ambr® 15 culture.

A decreasing glucose concentration implies that less glucose is added to the culture than consumed, which is necessary in the initial cultivation phase to achieve and maintain a target glucose level between 1–3 g/L, as high glucose levels induce higher lactate levels.[1,8,13–15]

The observations suggest that for the tested cell lines, the amino acid-to-glucose ratio in the feed was higher than the amino acid-to-glucose consumption ratio. Indeed, calculating the ratio of total amino acid consumption to glucose consumption revealed a much lower value than the ratio of total amino acid to glucose in the feed. Once the feed volume increased to maintain glucose between 1–3 g/L, the added amino acids exceeded the cultures' requirements, leading to increased relative amino acid concentrations. In the Ambr® 15 CHO-K1 cultivation, this effect was not observed, likely due to oxygen limitation and the resulting lower cell-specific glucose consumption rate.

Pan et al. (2017) observed an imbalance of amino acids relative to glucose in a rich medium | feed combination.[2] They identified different changes in the amino acid and glucose consumption rates of the cells upon the transition from growth to the stationary phase. The increasing amino acid concentrations in this study also correlated with the transition to the stationary phase, although no shift in glucose consumption was observed.

The rise in amino acid concentrations also correlated with increasing osmolality. Elevated osmolality can affect glycosylation by favoring higher mannose content.[3,16–18] In fact, an increase in mannose in the glycosylation profile of both 2 L cultures was observed over time, correlating with increasing osmolality. For future process optimization, reducing feed volume in the second half of cultivation—paired with separate glucose feeding—could help control osmolality and ammonium levels. This strategy can lower osmolality and ammonium concentration, optimizing the PQA profile by shifting to lower mannose glycosylation and higher galactosylation.

The PQAs in the 2 L cultivations changed significantly over time and in correlation with feed additions, emphasizing the potential to fine-tune PQAs through feed regime and process design. Additionally, reducing osmolality and ammonium concentrations may improve culture health, potentially resulting in increased cell growth and titer, as both high osmolality and high ammonium can inhibit cell growth.[1,11,19] In both cell lines, periods of higher osmolality corresponded to decreasing viability, although viability in CHO-S decreased only slightly at the end of cultivation.

In CHO-K1 cultures, the period of ceased cell growth and declining viability also corresponded to higher ammonium concentrations.

High ammonium concentrations can negatively impact cell growth and viability. Interestingly, in CHO-K1, the ammonium concentration decreased between Day 4 and Day 7 and started to increase again after Day 7 until Day 10. These findings correspond to changes in the cell growth rate during that time, indicating changes in the cells' metabolism.

Increasing osmolality also correlated with higher cell-specific productivity in the CHO-S culture, as higher osmolality can increase product titer.[13] The fact that osmolality in the bioreactor did not exceed levels observed in the Ambr® 15—despite significantly higher feed volumes—could be attributed to continuous feeding in the bioreactor compared with bolus feeding in the Ambr® 15 system. Continuous feeding has been shown to help maintain lower osmolality.[3,13] Furthermore, the higher VCD of CHO-S and the higher titers for both cell lines in the bioreactor could also contribute to lower osmolality.

Beyond the impact of high osmolality on protein glycan profile and charge variants, the analysis of trace elements in the spent media provided further insights into the process. CHO-K1 and CHO-S bioreactor cultures exhibited low concentrations of essential trace elements, like manganese and copper, from Day 7, and increasing zinc concentrations after Day 7. These trace elements can influence the glycan profile and charge heterogeneity.[17,20–25] Manganese is a cofactor for β-1,4-galactosyltransferase[26] and the ratio of manganese and zinc was shown to affect the galactosylation of proteins.[27]

Indeed, the reduction in complex glycans observed over time in both 2 L cultures correlated with low manganese and increasing zinc-to-manganese ratio. Notably, high osmolality, as observed in this study, can downregulate the transport of manganese.[28] and ammonia also affects galactosylation.[29] This underscores the importance of a holistic optimization of the cultivation process to fine-tune PQAs.

Conclusion

In summary, the glucose-based feeding strategy provided an adequate amino acid supply during the first half of the cultivation but led to overfeeding in the second half. Interestingly, the amino acid profiles were highly comparable between the Ambr® 15 and 2 L experiments. This demonstrates that amino acid analyses from Ambr® 15 screening experiments can be leveraged to optimize the feed regime in advance, highlighting the value of including spent media analysis early in process development. These results also underscore the advantage of using small-scale Ambr® 15 bioreactors to efficiently screen multiple conditions and generate reliable data for subsequent 2 L scale-up experiments.

An effective benchmarking process, as presented in this study, begins with the screening of different base media with FMA and FMB to identify the best combination for achieving a high titer, robust process, and correct PQAs. Samples from the optimal combination can undergo spent media analysis to optimize the feed regime in the next experiment, helping to avoid overfeeding, underfeeding, high osmolality, and accumulation of toxic byproducts. Scaling up to a 2 L bioreactor, or into an Ambr® 250, provides information about PQA profiles over time, enabling more in-depth analysis of spent medium, e.g., trace element analysis. Based on this data, further optimization of the process, feed regime, and feed formulation can be planned.

As off-the-shelf products are designed to fit a broad range of clones, the amino acid balance may not perfectly suit every clone. In a customized approach, amino acids with high concentrations—like glycine, phenylalanine, tyrosine, or valine—can be reduced, while those with lower concentrations—like asparagine—can be increased to achieve a better metabolic balance. This strategy is highly attractive for optimizing PQAs, cell culture performance, and titer. For instance, low asparagine concentrations in all cultures may have contributed to halted cell growth. Similarly, adjusting the concentrations of specific trace elements in the medium and feed, guided by spent media analysis, can further enhance product quality attributes. Data from studies like this enable a rational, cost-effective, and rapid adjustment of formulation and feed regime.

In summary, the extensive portfolio of Sartorius media and feeds can be effectively used to identify combinations that yield high titers. The advantage of off-the-shelf products lies in their immediate availability and validated performance. By employing the described screening strategy, the total time from pre-cultivation | adaptation to the completion of the 2 L benchtop bioreactor process can be reduced to just 10 weeks.

As outlined previously,[6] this strategy led to an effective initial feed regime without the need for testing multiple feed strategies. This procedure was successfully applied to two different cell lines, each with distinct nutrient requirements, using only one duplicate experiment per condition.

The accompanying analytics, especially amino acid, trace element, and PQA evaluations, allow scientists to further optimize the feed regime or explore a customized feed formulation with minimal effort and in a short time. This holistic approach is highly valuable, especially when time is critical, such as in the development of biosimilar processes.

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