SAFC®
Pharma & Biopharma Raw Material Solutions

White Paper

Turnkey Solutions to Improve Cell Culture Performance

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Optimizing media formulations and cell culture processes are essential for efficient and cost-effective upstream workflows. A wide variety of strategies can be applied during this phase of development to improve process economics, accelerate time to market, reduce regulatory and risk considerations, and ensure business continuity. This whitepaper describes two turnkey upstream solutions that can be applied to improve the performance of monoclonal antibody (mAb) production clones and set the stage for a successful manufacturing process. A scalable, simplified feed optimization process can be used to achieve high product titers, while incorporation of chemically defined supplements can mitigate risk and facilitate favorable product quality attributes.

One-Step Feed Optimization

Use of multiple feeds in the cell culture process increases complexity due to the need for extra manipulations and more connections between bioreactors and media bottles and bags. To enable a simplified one-step feed optimization, we have developed EX-CELL® Advanced CHO Feed 1 and Cellvento® 4Feed, which are part of our fed-batch media and feed platform systems. Both systems are chemically defined and suitable for a diverse set of CHO cell lines grown in suspension.

EX-CELL® Advanced CHO Feed 1 contains a high concentration of key components and was developed using multivariate analysis and data mining to establish correlations between raw materials, and critical process and product attributes. Cellvento® 4Feed is a highly concentrated feed (>130 grams per liter), which is reconstituted at neutral pH. Incorporation of modified amino acids and amino acid derivatives supports the slow release of amino acids such as cysteine and tyrosine, which is advantageous to the culture process. Additionally, modified amino acids in the feed design allow for reconstitution in a single preparation under neutral pH, eliminating the need to manage multiple feed additions prepared under different pH values and fed separately into the culture. Furthermore, this reduces the risk of contamination and simplifies the process.

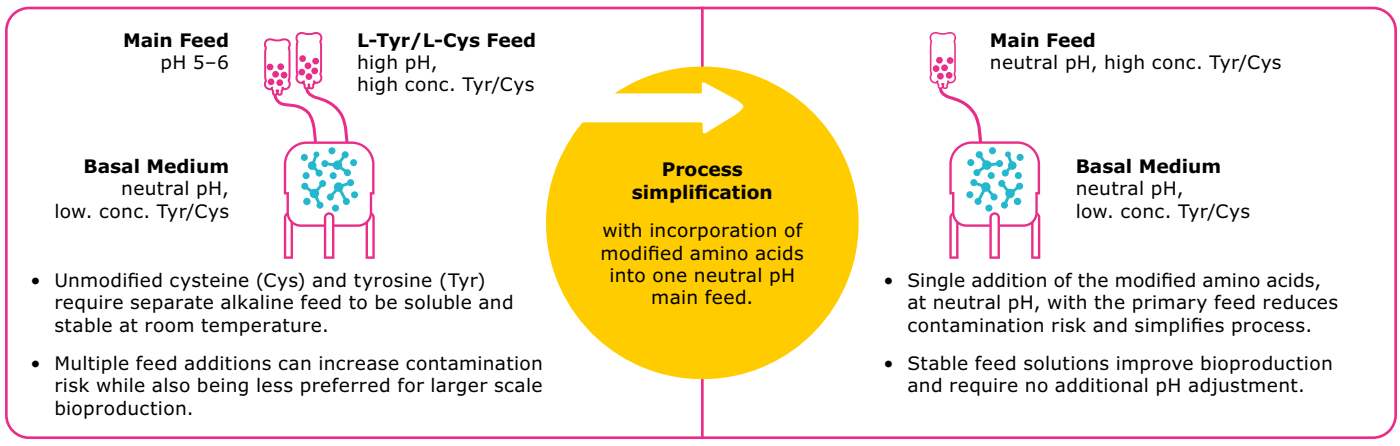


Figure 1.

Chemically-modified tyrosine and cysteine enable a single feed strategy at neutral pH.

Importantly, EX-CELL® Advanced CHO Feed 1 and Cellvento® 4Feed can be combined and fed as a simple unit operation; each feed is hydrated individually and mixed as per the desired ratio. With this one-step feed optimization solution using feed blends, the diversity of feeds being screened is increased and can deliver a twofold increase in titers for some production clones.

Figure 2 shows the viable cell density and IgG titers from a one-step feed optimization for three clones, from an IgG cell line development project (designated mAb01), generated using the CHOZN® GS CHO cell line development platform with UCOE® expression technology (CHOZN® & UCOE® combined platform). In addition to the individual EX-CELL® Advanced Feed 1 and Cellvento® 4Feed formulations, a single feed blend (50:50) was evaluated which consisted of 50 percent of each feed. Clone A preferred the 50:50 feed blend as evidenced by the higher viable cell densities and titers, whereas clones B and C had similar performance in all three conditions.

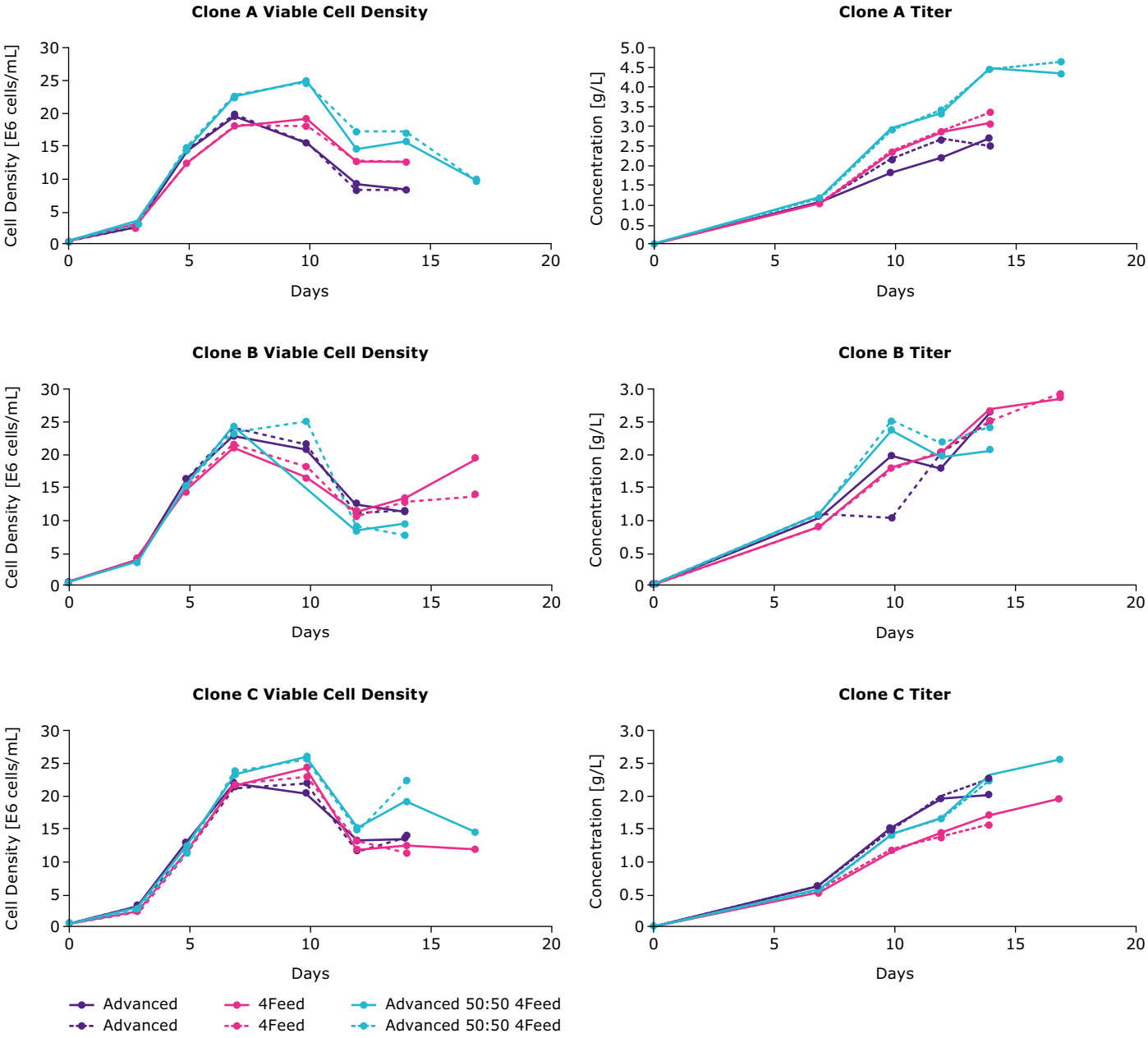


Figure 2.

One-step feed optimization for mAb01 fed-batch cultures in spin tubes.

Clone A and B and the best-performing 50:50 feed blend were subsequently scaled to 3 L and 50 L Mobius® bioreactors and showed comparable results to the spin tubes data, demonstrating the scalability of the feed blend (Figure 3).

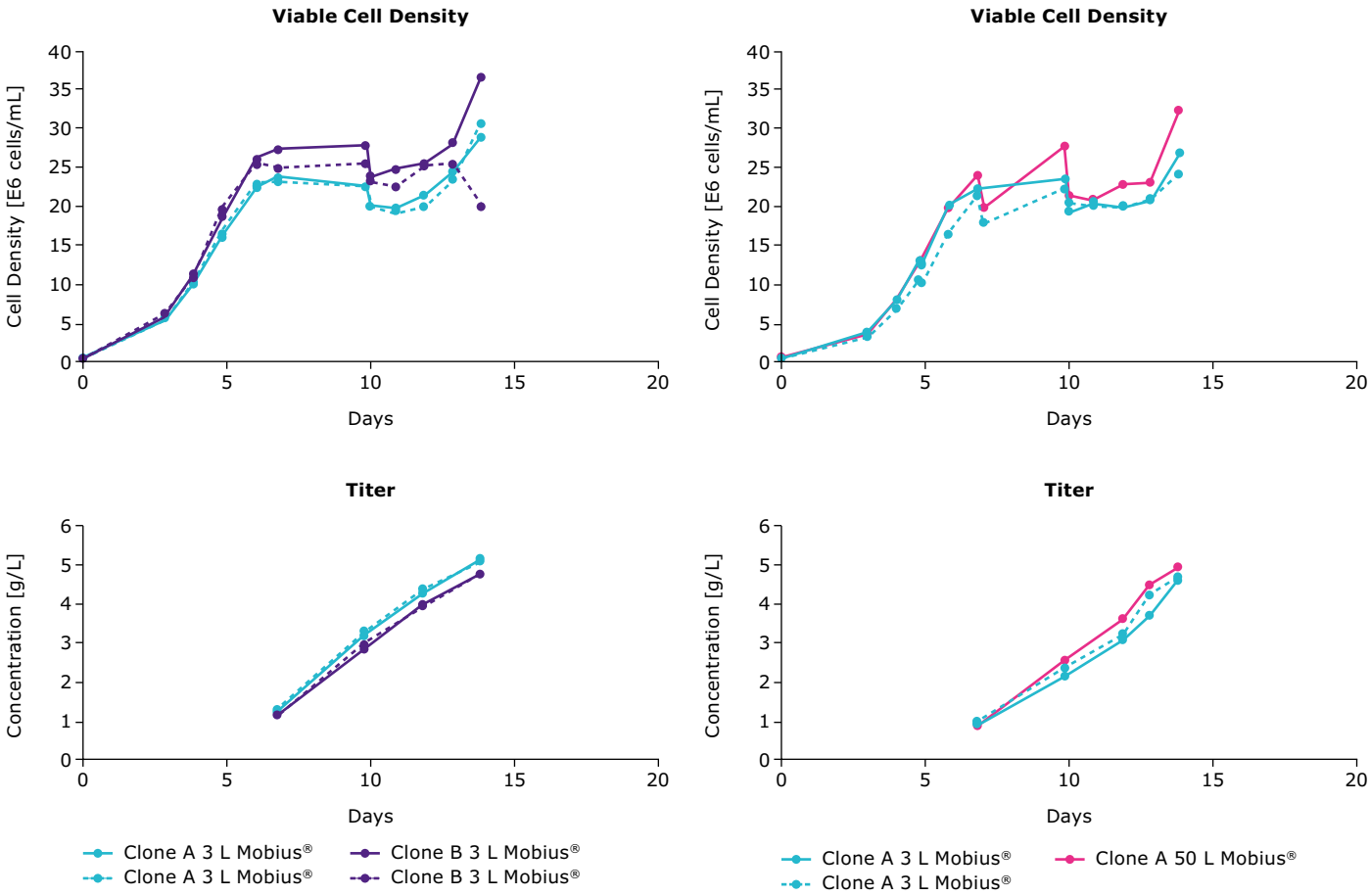
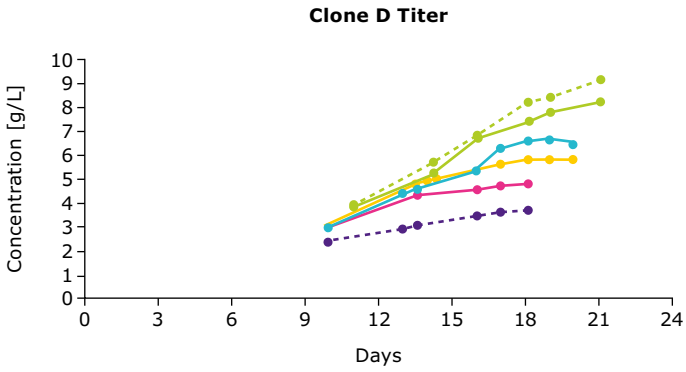
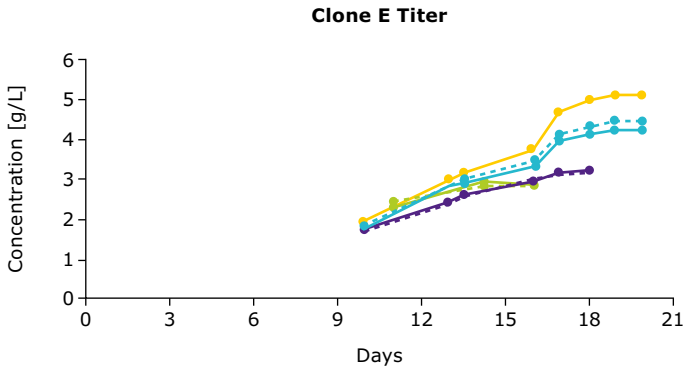


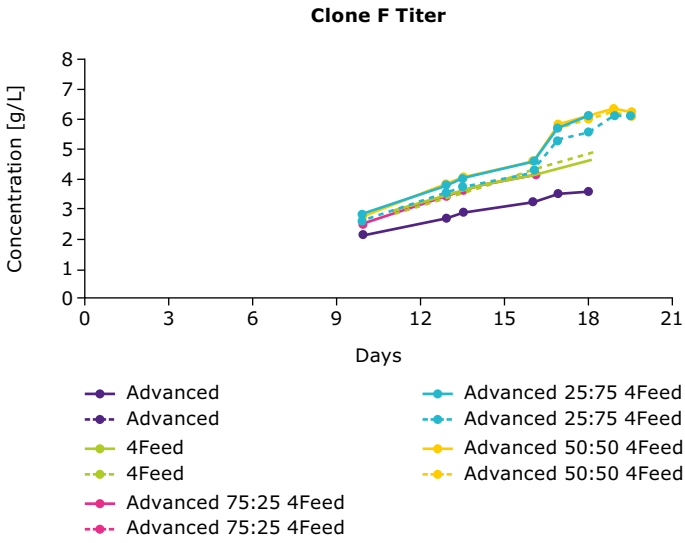
Figure 3.

Viable cell density and titers from mAb01 clones A and B in 3 L and 50 L Mobius® bioreactors.

Figure 4 summarizes results from the one-step feed optimization for three clones from another IgG cell line development project (mAb03) generated with the CHOZN® & UCOE® combined platform using three feed blends. The clones preferred different feed blends and the mechanism of performance improvement varied from clone to clone. For example, clone D preferred the individual Cellvento® 4Feed whereas clone E preferred the 50:50 feed blend; clone F preferred both the 50:50 and the blend with 75 percent Cellvento® 4Feed (75:25). In terms of mechanisms, clone D and clone E showed improvement in viable cell density leading to higher titers. For clone F, improvement was observed in specific productivity.





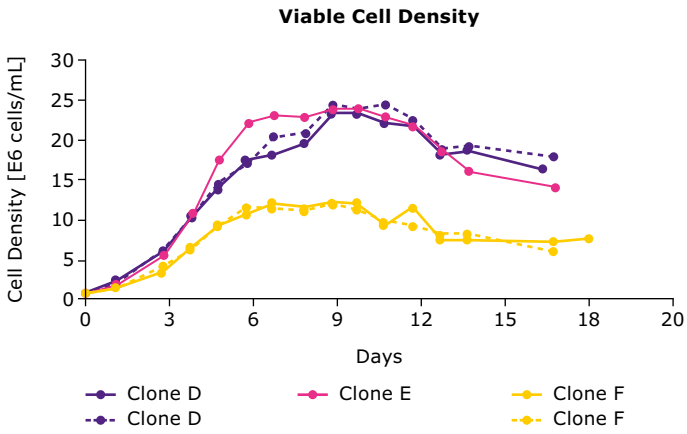


	Clone D		Clone E		Clone F	
Feed	Peak Cell Density X 1E6	Titer (g/L)	Peak Cell Density X 1E6	Titer (g/L)	Peak Cell Density X 1E6	Titer (g/L)
Advanced	18	3.7	17	3.2	9	3.5
Advanced 75:25 4Feed	20	4.8	19	3.2	9	4.0
Advanced 50:50 4Feed	23	5.8	21	5.1	10	6.1
Advanced 25:75 4Feed	28	6.5	24	4.4	9	6.1
4Feed	25	8.7	24	2.9	9	4.7

Figure 4.

One-step feed optimization for mAb03 fed-batch cultures in spin tubes.

A similar scale-up experiment was performed for mAb03 to demonstrate scalability of the individual feed, Cellvento® 4Feed (Figure 5). The peak viable cell density and titer of the three clones in the 3 L Mobius® bioreactors was similar to that observed in the spin tubes screening process.



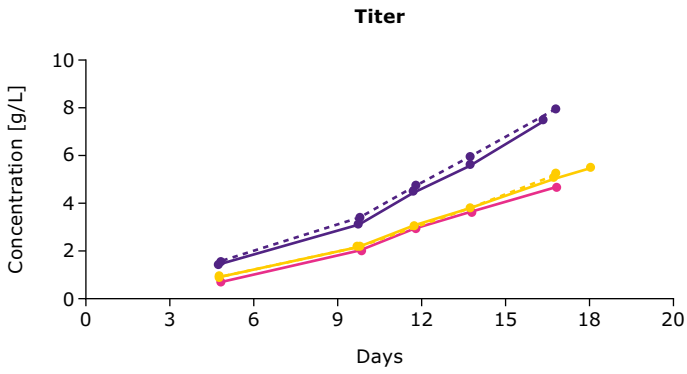


Figure 5.

Viable cell density and titers from clones of mAb03 in 3 L Mobius® bioreactors.

The one-step feed optimization process can also be used with difficult to express proteins such as bispecific antibodies. Screening results, for clones generated with the CHOZN® & UCOE® combined platform, with two feed blends and the individual feeds along with subsequent scale-up for three clones with the best performing feed blend (67:33) are shown in Figures 6 and 7, respectively. As with the previous results, these clones required different feed blends to achieve the highest titer (Figure 6) and the process was scalable (Figure 7). Titers of 2 to 3.5 g/L for the bispecific antibody were achieved in a 3 L Mobius® bioreactor using the best performing feed screened during the one-step feed optimization.

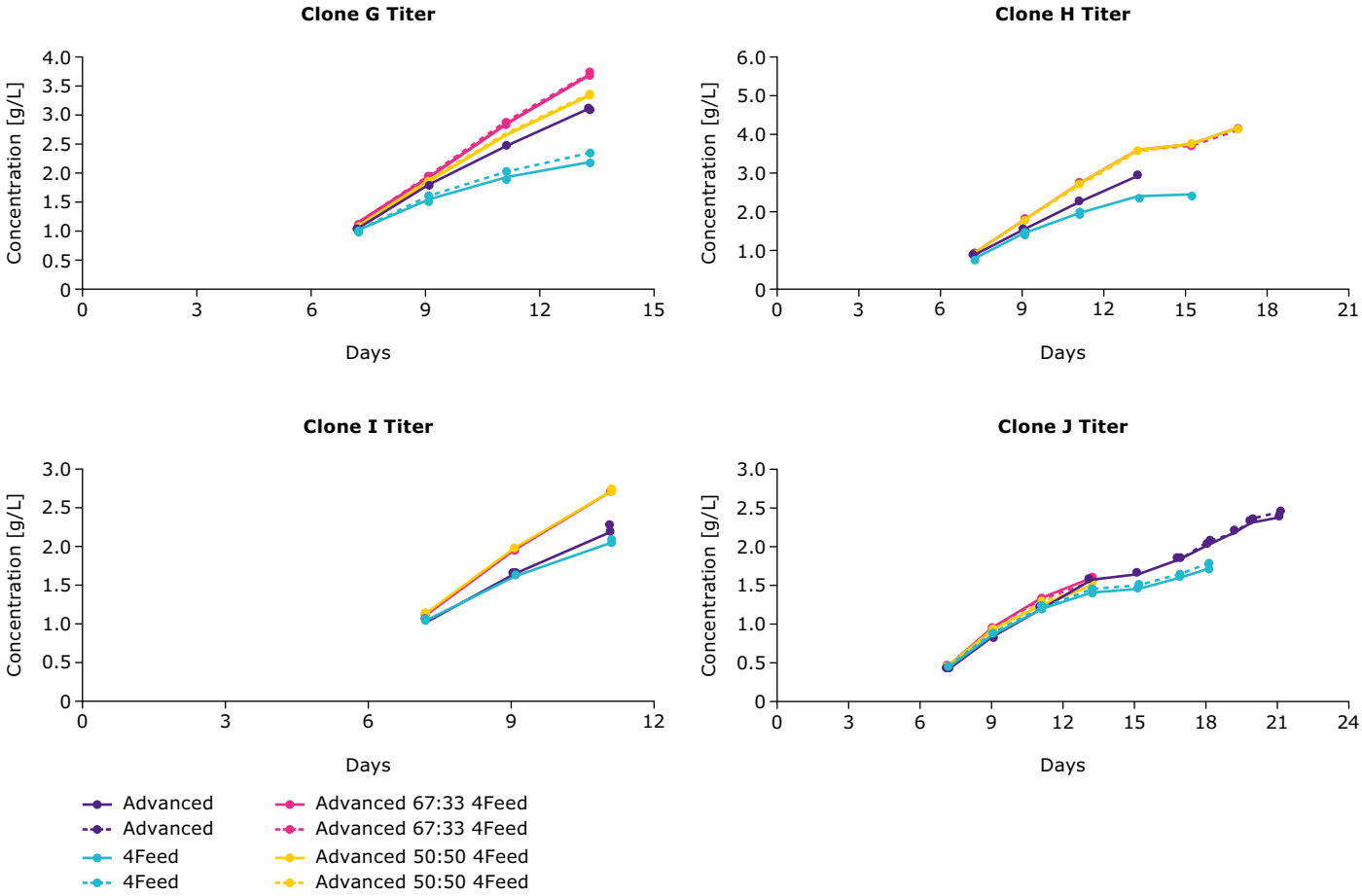


Figure 6.

One-step feed optimization for bispecific antibody fed-batch cultures in spin tubes.

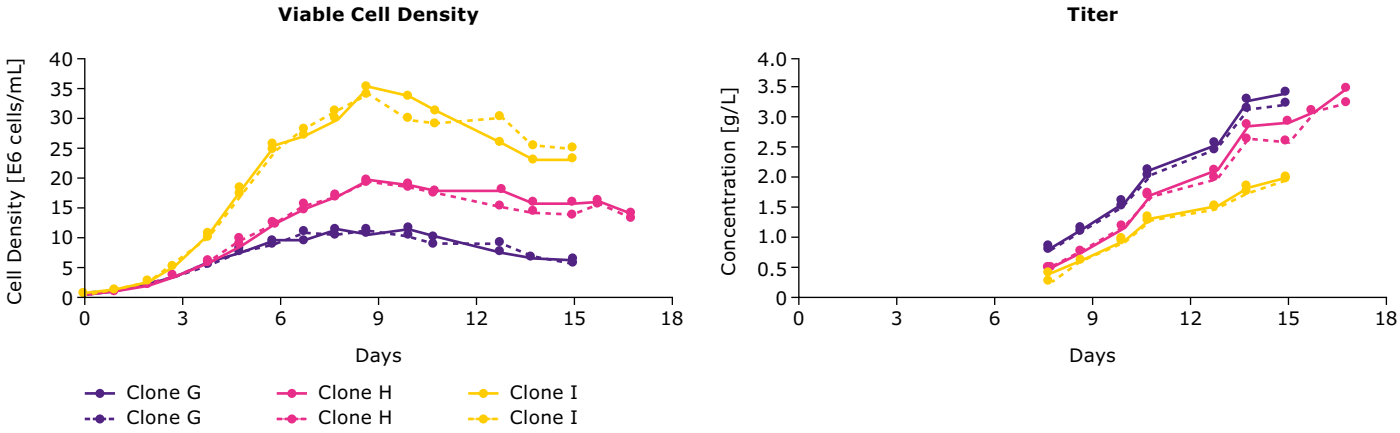


Figure 7.

Viable cell density and titers of bispecific antibody clones in 3 L Mobius® bioreactors.

The data shown reinforces that different clones prefer different feed blends, and the mechanism of performance improvement varies from clone to clone. To further explore these observations, a heat map based on the main nutrient components in the feed blends was developed; components were grouped as amino acids, vitamins, lipids, trace metals, and other components along with a total concentration classification (Figure 8).

The different feed blends offer a diversity in nutrient components, where some blends may be lower in certain nutrients but higher in others. Given this diversity, it is advisable to screen all the feed blend ratios to increase the coverage and probability of finding a feed that is preferred by the clone for optimized performance.

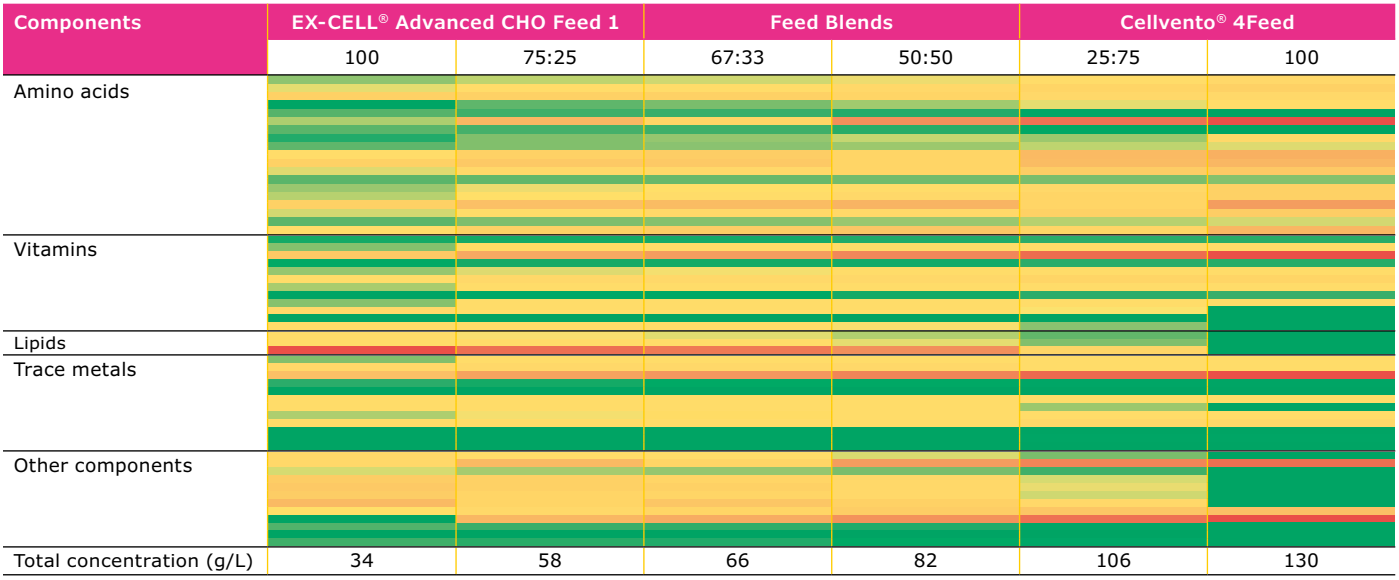


Figure 8.

Heatmap describing EX-CELL® Advanced, Cellvento® 4Feed and their blends.

Legend

Low — High

Chemically Defined Media Supplements

In addition to the use of scalable and simplified feed optimization to achieve higher titers, chemically defined (CD) supplements can be used to reduce risk and facilitate favorable product quality attributes.

Hydrolysates are commonly used in cell culture media as a supplement to enhance cell growth and recombinant protein productivity and contain diverse classes of compounds, such as peptides, carbohydrates and phenolic compounds. Replacement of undefined, naturally derived hydrolysates with a chemically defined alternative product reduces lot-to-lot variation and the risk of contamination. To develop this chemically defined alternative, various natural hydrolysates were fractionated by reversed phase HPLC; fractions were screened in CHO cell cultures with a base supplement that contains easily identified hydrolysate components. The fractions which had a significant impact on the cultures were identified and chemically defined versions were sourced. The chemically defined alternatives were screened with multiple CHO cell lines and a final combination was created with the base supplement, which is now available as EX-CELL® CD hydrolysate fusion.

Figure 9 compares the impact of the EX-CELL® CD hydrolysate fusion and natural hydrolysates on viable cell density and titer in a batch process. The data shows that EX-CELL® CD hydrolysate fusion is comparable to the undefined, natural product.

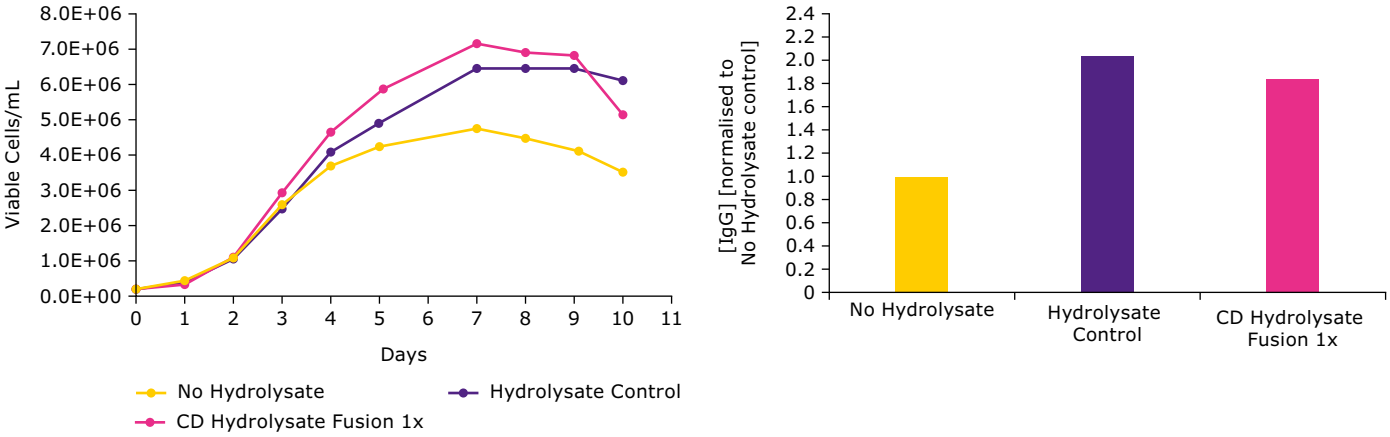


Figure 9.

Comparison of chemically defined and natural hydrolysates on viable cell density and productivity in a batch process.

Figure 10 shows the results of a study in which the EX-CELL® CD hydrolysate fusion was used in place of natural hydrolysates in a batch study with a different CHO clone. Growth performance and titer production was comparable to the undefined natural hydrolysate-containing culture conditions. These data demonstrate that the EX-CELL® CD hydrolysate fusion is a good alternative to replace undefined hydrolysates while maintaining or achieving higher titers and reducing contamination, lot-to-lot variability, and process variability risks.

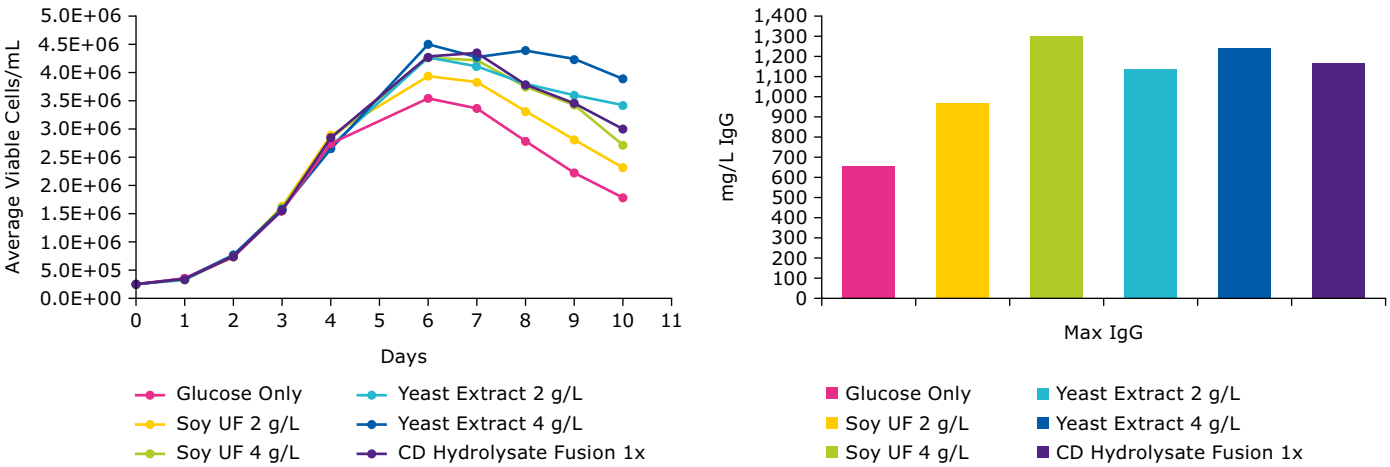


Figure 10.

Comparison of EX-CELL® CD hydrolysate fusion and natural hydrolysates in a batch process.

Chemically defined supplements can also be used to facilitate favorable product quality attributes such as glycosylation which affects the structure, folding ability, stability, *in vivo* half-life, immunogenicity, and efficacy of a drug molecule. Understanding the glycan profile is especially critical for biosimilars which must demonstrate comparable physiochemical, structural, and functional properties, as well as similar efficacy, immunogenicity, and safety profiles to the originator molecule. The glycan profile can be determined by various parameters including the cell line, vector designs, and how clone screening and selection are conducted. Process parameters such as temperature, pH, dissolved oxygen, media, and supplements can also affect glycan profiles. A solution for rapid modification of glycan profiles is the use of a targeted protein quality supplement. The specific purpose of said supplement being to increase galactose site occupancy in order to achieve functional shifts in N-linked glycosylation quickly and efficiently.

Two experiments were conducted to illustrate how a supplement can help to shift glycan profiles. The first was conducted using fed-batch culture in spin tubes with three different mAb-producing CHO cell lines (CHO-M, DuxB11, and CHOZN® GS) cultured with and without the EX-CELL® Glycosylation Adjust supplement (GAL+). A second experiment evaluated scalability of supplementation with GAL+.

Figure 11 shows the glycan distribution profiles from both experiments. Two-to-four fold increase in the relative G1F and G2F distribution were observed both in the fed-batch culture in spin tubes, across the range of CHO cell lines, and during the subsequent scale up of the CHOZN® GS cell line to the 3 L Mobius® bioreactor; all three CHO cell lines showed a similar shift towards G1F and G2F. There was no change in growth and titer profiles (data not shown) indicating that the protein quality supplement targets the glycan shift and does not affect growth or titer.

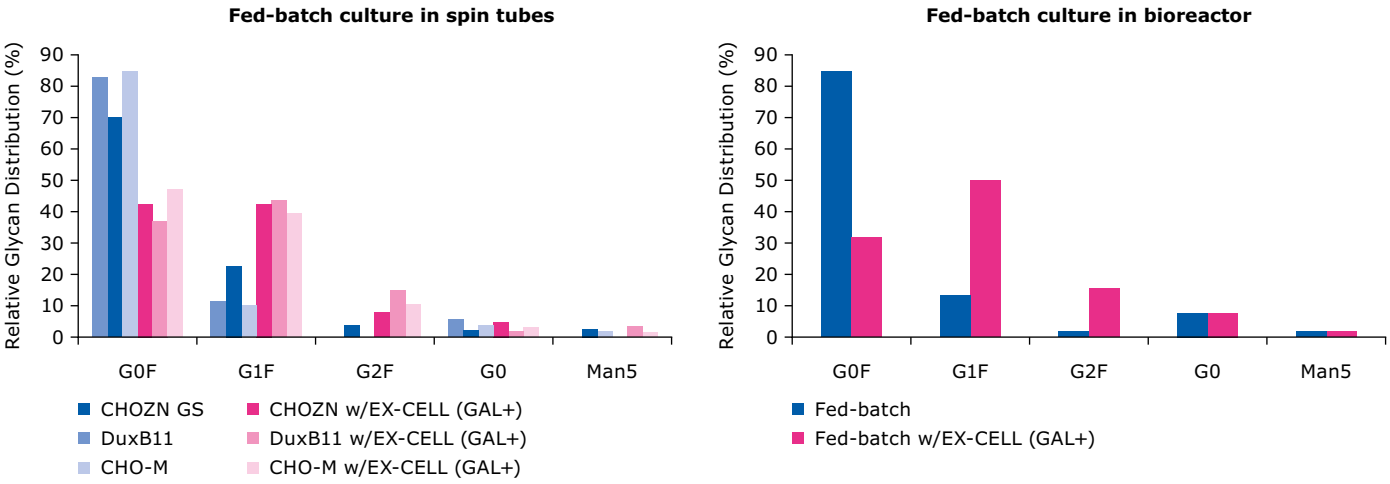


Figure 11.

Change in relative G1F and G2F distribution using the GAL+ supplement.

Titer goals must be balanced with targeted glycan profiles and both can be affected by process parameters such as temperature, pH, and dissolved oxygen. Figure 12 summarizes a study in which a process was designed to first increase titer and resulted in a shift of the glycan profile. A temperature shift to 34 °C was initiated on day 6 and continued to day 14. While there was an increase in titer from 1.5 g/L to 2 g/L, the process resulted in an increase of G0F. With GAL+ supplementation, the glycan profiles were shifted without affecting the titer improvement achieved with the earlier process optimization. This study confirmed that GAL+ supplement can be used together with temperature shift to achieve an increase in titer, G1F and G2F.

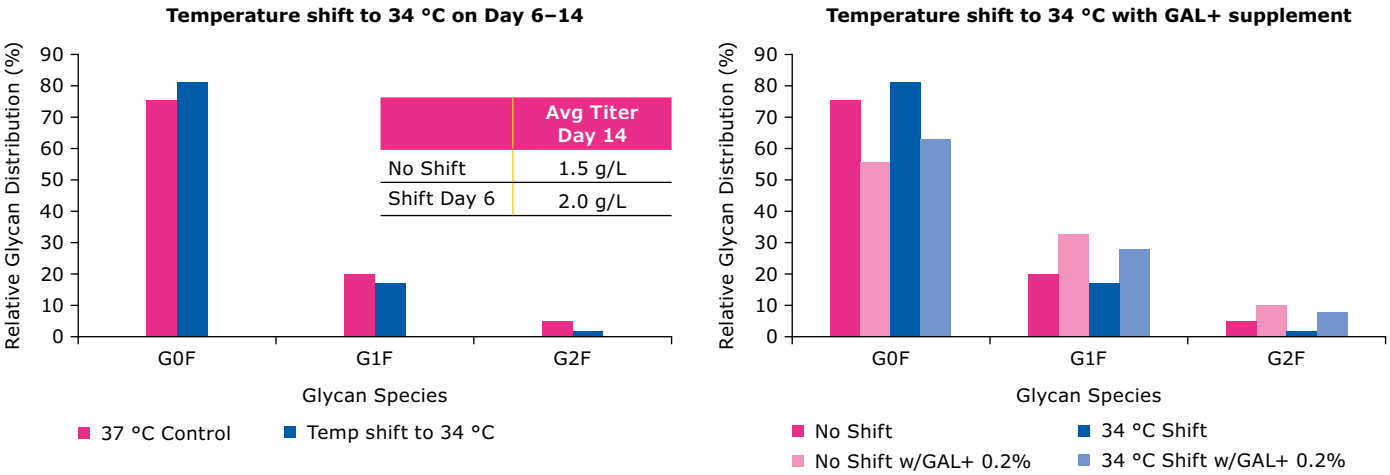


Figure 12.

Use of GAL+ supplement to balance titer and glycosylation requirements.

Conclusion

The ability to optimize scalable upstream processes sets the stage for successful clinical- and commercial-scale production. This whitepaper demonstrated application of two strategies (one step feed optimization and use of chemically defined media supplement) that not only optimize upstream processes but can also mitigate risk and reduce time to market.

Platform feed screens using feed blends were shown to accelerate and simplify media and process development, while a chemically defined hydrolysate fusion can reduce the risk in raw materials, lot-to-lot variation, process variations, and possible contamination. Use of a novel targeted protein quality supplement was shown to increase galactose site occupancy, modifying N-glycan profiles across a broad range of CHO cell lines.

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