Development of BD Resurge™: A Family of Chemically Defined Cell Culture Formulations for Use as Bioprocess Supplements and Feeds

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ABSTRACT

Optimization of cell culture parameters is an essential component of process development for production of active biopharmaceutical ingredients. Every cell line offers unique challenges for developing an optimal cell culture process that yields desired growth profiles, titers, and product quality. Different growth conditions are typically attempted before process goals are met. A significant portion of the optimization process is committed to determining cell culture media and feed conditions that will boost performance. BD Resurge™ CD supplements, a diverse set of chemically defined (CD) formulations for use as supplements or feeds in CHO culture systems, were developed to facilitate this process. This paper demonstrates that BD Resurge CD supplements can enhance culture performance and antibody production in multiple CHO cell lines while maintaining N-glycan profiles. Additionally, scalability is demonstrated by the consistent performance of the CD supplements in both shake flasks and bench-top bioreactor cultures. BD Resurge CD supplements provide varied options for media supplementation to help reduce the timelines associated with cell culture process development.

Key words: Cell culture, multiple cell lines, feed, supplement, chemically defined, scalability, active pharmaceutical ingredients, product quality, optimization.

INTRODUCTION

Cell-based production of biotherapeutic proteins generally requires supplementation of cell culture media to promote cell growth and boost titer. Every cell line, clone, and process has unique biological requirements to stimulate growth and protein production. Therefore, the paradigm of "one-supplement-fits-all" is not easily attainable in a biopharmaceutical process. The primary challenge facing process development scientists is the selection and optimization of a cell culture supplement or feed that will give the desired titer, growth characteristics, and protein quality. The availability of multiple Chemically Defined (CD) supplements or feeds offering a diverse performance profile can significantly reduce process development timelines.

BD Resurge™ CD PAK, a CD and animal-free (AF) cell culture supplement kit, was developed to help address these challenges. The overall steps used to develop this diverse set of CD supplements are outlined in Figure 1. The availability of the five BD Resurge formulations as a pack facilitates the testing of multiple CD supplements under different process conditions in a single design of experiments (DOE) study, thereby increasing the likelihood and speed of identifying the media and supplement/feed pair required for optimal performance.

The BD Resurge CD supplements can be used in batch and fed-batch processes to enhance recombinant protein production of CHO cells while maintaining suitable product quality. Best results can be attained by using supplements at optimized concentrations and feeding times. These supplements have been tested on a variety of CHO cell lines and in various culture systems, including shake flasks, microbioreactor (ambr™) and bench-scale bioreactors.

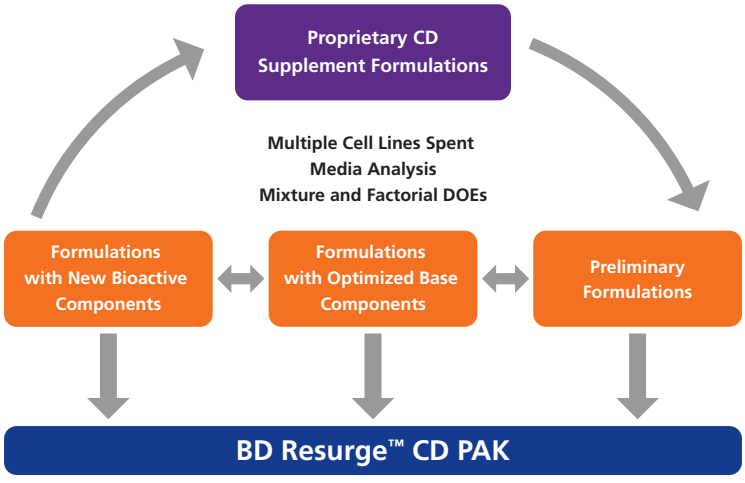


Figure 1: BD Resurge CD PAK Development Process

MATERIALS AND METHODS

Bioassay

Bioactivity of CD components and formulations were tested using several CHO cell lines: CHO-K1 (CHO line 1 and 2), GS-CHO (CHO line 3), and CHO-DG44 (CHO line 4). These CHO cell lines stably express monoclonal IgG antibodies (mAbs). Screening was performed in batch mode and fed-batch studies were performed after finalizing the formulations. For batch cultures, supplements were added on day 0 of culture and glucose was maintained at 3 g/L throughout. Cell culture assays were carried out in 125mL shake flasks, seeded at 2-3 x 105 cells/mL in a shaking, humidified incubator set at 5% CO2 saturation and 125 RPM. DASGIP® and New Brunswick™ bench-scale bioreactors (Eppendorf®) were used for confirmation studies at 1L working volume. Ambr™ microbioreactor (Sartorius Stedim) was used to model bioreactor conditions at micro scale (15 to 17mL working volume) under controlled conditions. Cultures were sampled for assessment of cell growth and protein production on various days. The viable cell density (VCD) and viability were determined using a Beckman Vi-Cell® (Beckman Coulter). Protein titer was determined using Protein A biosensors on a ForteBio Octet® system (Pall Life Sciences).

Spent Medium Analysis

Spent medium analysis was performed with a Waters ACQUITY™ UPLC® System using Waters AccQ•Fluor™ methodology (amino acids), a Waters ACQUITY UPLC-TQD system (bioactive small molecules) and an Agilent 7500cs ICP-MS system (elemental analysis) equipped with the Octopole Reaction System (ORS). Component classes were then selected for optimization of base formulations.

Protein Quality

Using spent media from batch mode supplemented cultures, monoclonal antibodies were purified using Protein A chromatography. The N-glycan profiles were analyzed by a HILIC-ACQUITY UPLCTM chromatographic method (Waters). Fluorescent-labeled N-glycan pools released from the monoclonal antibodies (mAbs) were identified by comparison to a reference sample.

RESULTS AND DISCUSSION

Proprietary Mixture DOE Study

We used a DOE approach to generate mixtures based on three proprietary base supplement formulations (A, B, and C). A simplex centroid design was utilized to generate ten mixtures from the three base formulations. These initial mixtures were tested on multiple cell lines in basal CD media to determine the impact on growth and titer. As shown by the contour plots in Figure 2, mixtures composed of a higher proportion of formulations A and C gave relatively better VCD than other mixtures, whereas combinations with a higher percentage of formulations A and B gave comparatively better viability. No single mixture was optimal for VCD, viability, and titer. Therefore, mixtures with suboptimal responses for all parameters were selected for further optimization.

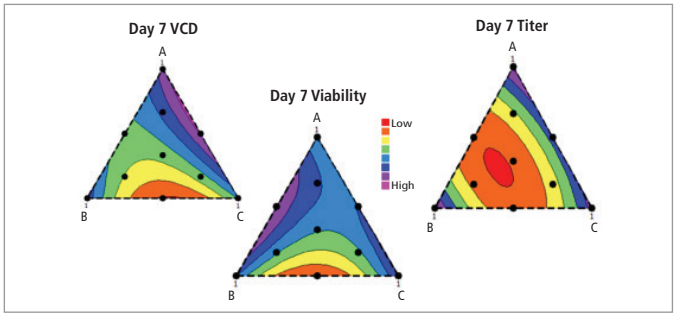


Figure 2: Proprietary mixture DOE study. Contour plots show VCD, viability, and titer responses in a simplex centroid mixture design with three proprietary formulations (A, B and C). Black dots represent mixture points.

Base Component Optimization

Based on analysis of spent media, seven component classes were tested in a fractional factorial design study. Each component class was tested at two concentrations, which generated a total of 70 supplement mixtures. Three different host CHO cell lines were tested in batch culture. The supplement mixtures induced a broad range of response profiles, with many mixtures showing enhanced performance in one or two cell lines, but not in all three. A representation of the sample mixtures is shown for two cell lines (Figure 3). In CHO line 2, a CHOK1 host, the responses were more scattered, demonstrating noticeable differences in performance of some mixtures, whereas in CHO line 3, a GS CHO host, the results from all 70 mixtures were more tightly clustered, showing comparable performance for protein titer and VCD. Similar observations were made using CHO line 4, a DHFR host (data not shown).

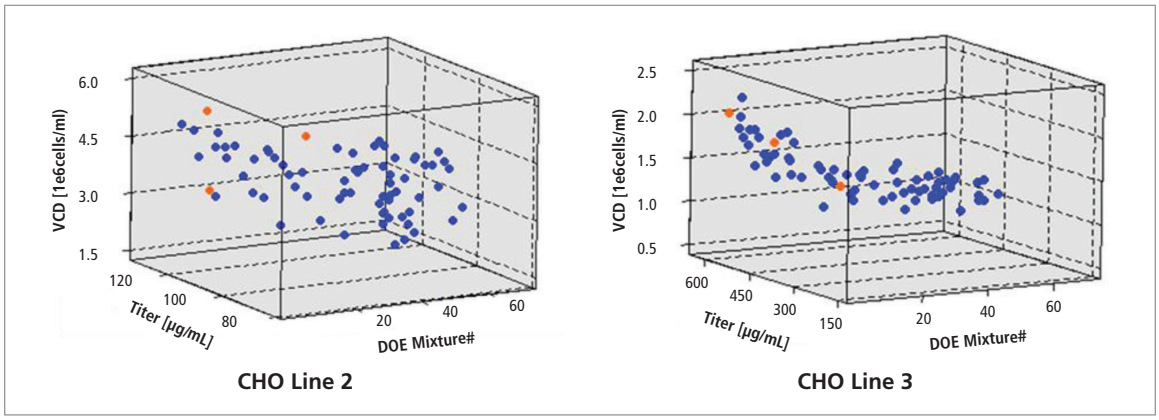


Figure 3: 3-D scatterplots showing growth and production results for 70 DOE mixtures evaluated in two cell lines. Mixture numbers are shown on x-axis, titer in µg/mL on y-axis, and VCD in 1x 106 cells/mL on z-axis. A subset of mixtures (2, 17, and 33) is displayed as orange points to illustrate the distinct responses of each CHO cell line to these specific mixtures.

Bioactive Component Addition

During the initial round of optimization, some classes of components were identified as bioactive, since their presence in some of the mixtures led to significant improvement in performance in all three cell lines tested. Initial candidate formulations were further optimized by addition of these bioactive components. Performance of bioactive components was dependent on base medium formulation and cell line. For example, the effect of addition of a class of bioactive components to two top-performing supplement formulations (D and E) was tested in two different cell lines. Addition of one class of bioactive components to CD supplement D for CHO line 1 in a batch culture study improved cell viability, longevity, and titers compared to original supplement control (Figure 4A). When the same class of bioactive components was added to CD supplement E for CHO line 3 in a batch culture study, protein titer was improved while growth and viability were unaffected (Figure 4B). These data confirmed that distinct bioactive components may be required to stimulate different cell growth characteristics in each cell line. Therefore, further optimization of potential CD supplement candidates involved titrating classes of bioactive components identified during multiple DOE studies to achieve optimal cell growth characteristics (titer, VCD and viability) across multiple cell lines.

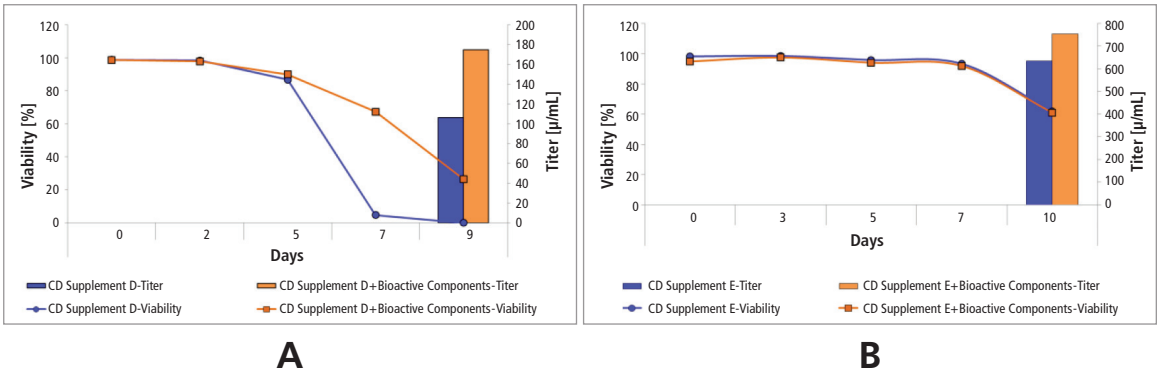


Figure 4: Bioactive component addition to CD supplement D with CHO line 1 (A) and to CD supplement E with CHO line 3 (B).

Performance Across Multiple Cell Lines

Following multiple rounds of optimization, the top seven CD supplement formulations were selected and tested across multiple CHO cell lines to assess their versatility in enhancing performance. Spin tube and shake flask formats were used in these development studies and each cell line was tested using its respective control base medium.

The cell lines showed varying responses to all seven CD supplements (S1-S7) (Figure 5). In general, most of the supplements increased protein titer over the control base media, with significant increases (three-fold and above) observed in CHO line 3 supplemented with S4, S5, and S6. All supplements, except S7, led to a significant increase in integral viable cell density (IVCD) for CHO line 2. These data illustrate the importance of testing a wide range of supplements during product development, as each cell line requires a unique medium environment (base medium plus supplement or feed) for optimal performance.

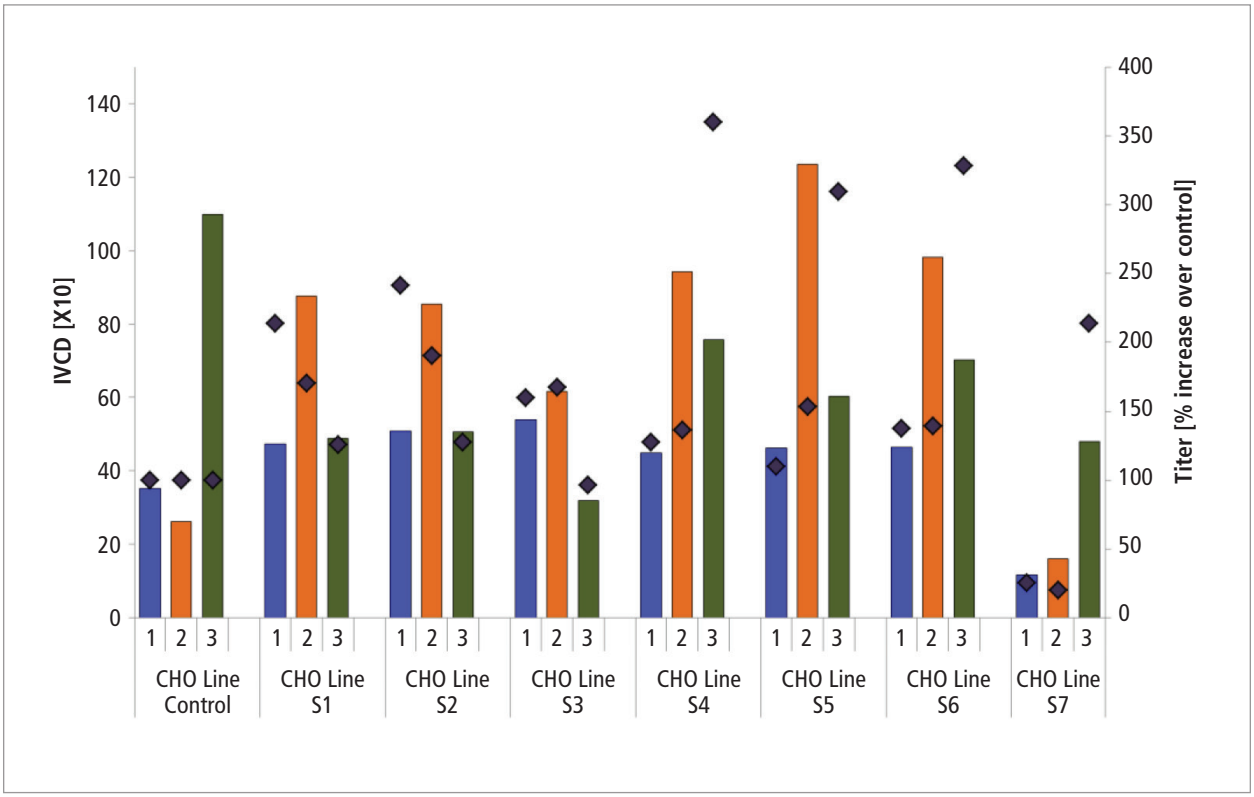
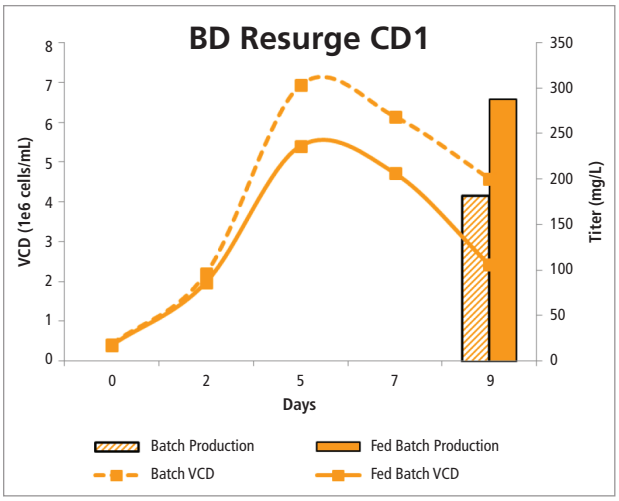


Figure 5: Differential CD Supplement Performance in Multiple Cell Lines. Integral viable cell density (IVCD; columns) and titer (% increase over control; diamonds) of individual supplements (S1-S7) on different cell lines in a batch culture.

The final five formulations (BD Resurge CD 1 – 5) were selected based on overall performance across the cell lines tested. To demonstrate that BD Resurge CD supplements can be used as supplement and as feed, the formulations were tested in shake flask in batch (BD Resurge CD supplement added to base medium on day 0) and fed batch modes. Optimal feeding strategies were also established for each BD Resurge CD supplement. A representative data set for BD Resurge CD 1-2 in CHO line 1 and BD Resurge CD 3-5 in CHO line 3 is shown in Figure 6. Feeding of BD Resurge CD supplements resulted in increases in protein titer over batch cultures (Figures 6A-E). The VCD for fed-batch mode was comparable to the corresponding batch culture.



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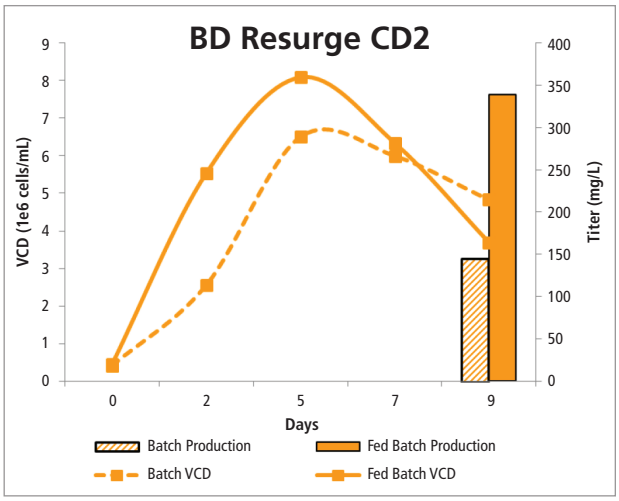

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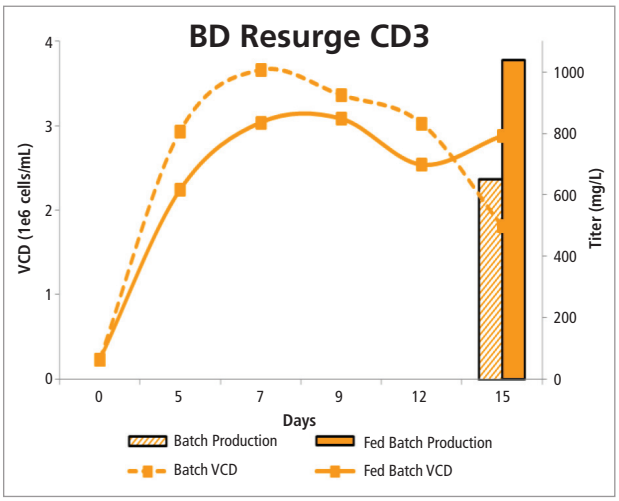

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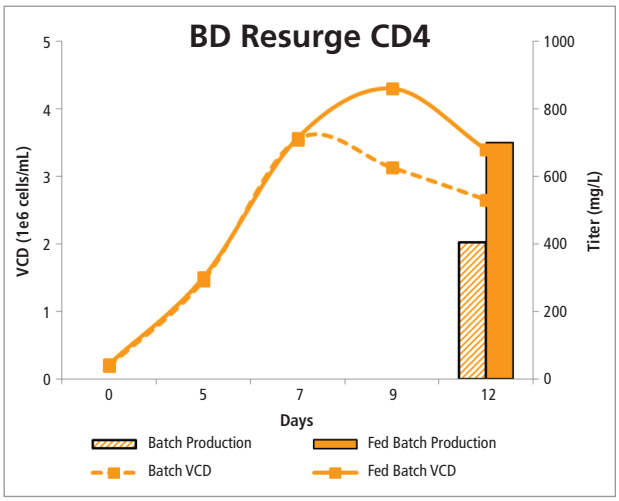

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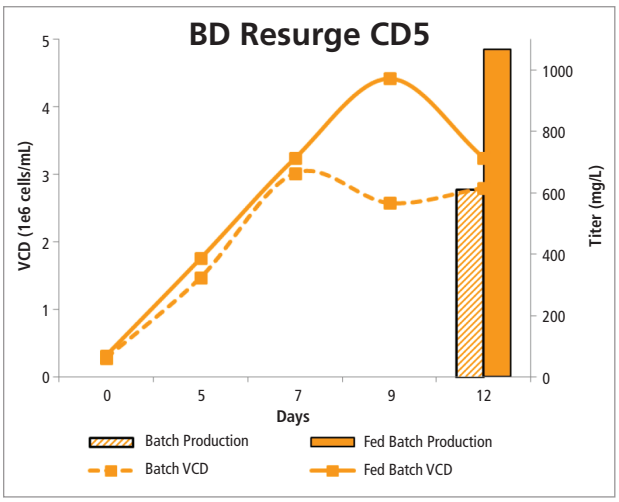
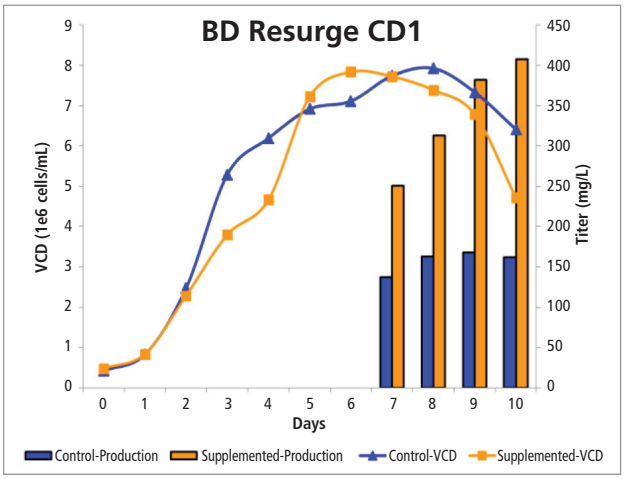


E

Figure 6A–E: Growth and titer of CHO cells cultured with BD Resurge CD supplements in batch and fed-batch modes. Cultures were fed on days 0, 2 and 5 for CD1 (A) and CD2 (B); on days 0, 5 and 7 for CD3 (C); and on days 0, 3, 5 and 7 for CD4 (D) and CD5 (E).

Scale-up Studies

BD Resurge CD 1-5 were evaluated across multiple culture systems. All supplements elicited a 2- to 3-fold increase in mAb titer over base medium only control in bench-scale bioreactors in batch or fed-batch mode following process optimization (Figure 7). Similar results were obtained in shake flask studies (Table 1). Optimal concentrations for each BD Resurge CD supplement were used in the batch culture study. Together, the results demonstrate that the performance of BD Resurge CD supplements was scalable from shake flask to bench-scale stirred-tank bioreactor.



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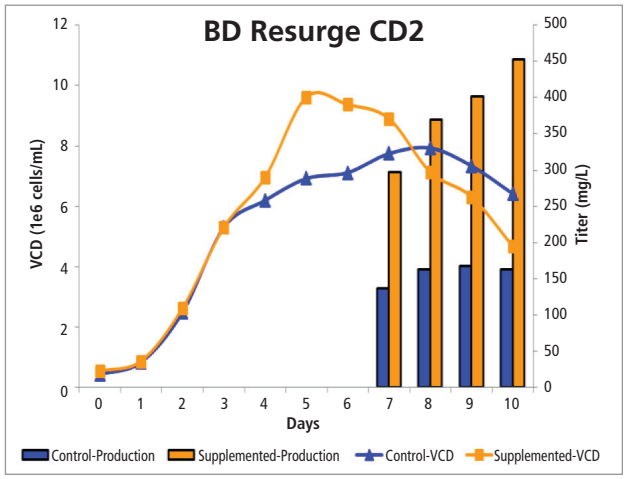

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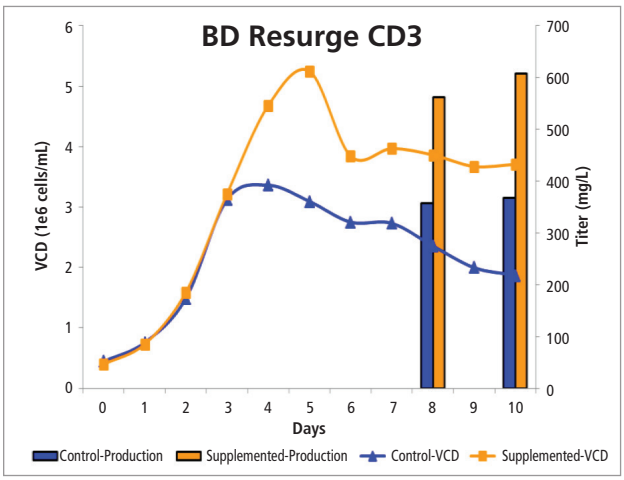

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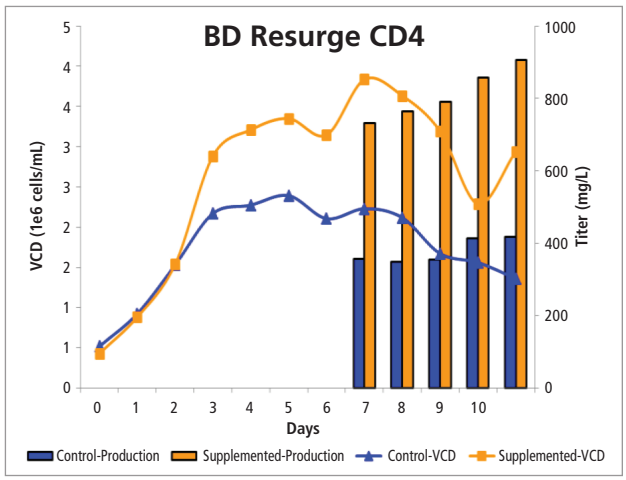

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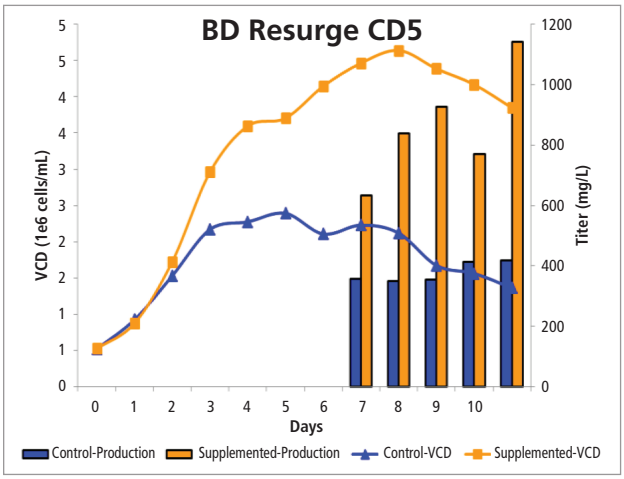

E

Figure 7A–E: BD Resurge CD PAK (CD1-5) in a bench-scale bioreactor system. CHO line 1 was used with BD Resurge CD1 and CD2 (fed batch mode), while CHO line 3 was used with BD Resurge CD4-CD5 (batch mode). CHO line 3 was used for BD Resurge CD3 in ambr micro bioreactor in batch mode.

Medium/Supplement	Cell Line	Fold Increase Over Medium Only
Medium Only	CHO line 1 or CHO line 3	1
BD Resurge CD 1	CHO line 1	2.7
BD Resurge CD 2	CHO line 1	3.6
BD Resurge CD 3	CHO line 3	2.0
BD Resurge CD 4	CHO line 3	1.9
BD Resurge CD 5	CHO line 3	3.0

Table 1: Fold Increase in Protein Titer in Shake Flask studies

Protein Quality

The N-glycan profiles were analyzed for mAbs produced in medium-only controls and BD Resurge-supplemented conditions in two cell lines. The relative compositions of the most prevalent glycans from each cell line are depicted in Figure 8. The identified glycans are labeled with the corresponding abbreviation. In CHO line 3 samples, glycans not identified are numbered. Differences between the supplemented and control conditions were similar to those seen when using other legacy supplements, such as peptones (data not shown).

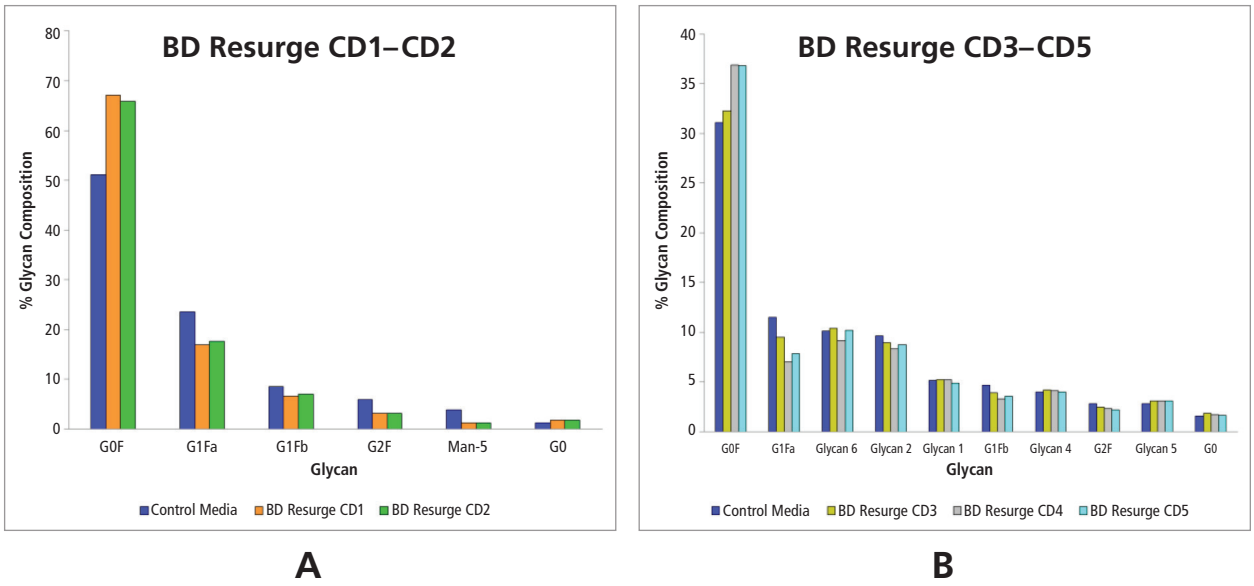

Figure 8A–B: The N-glycan composition of mAbs produced in each supplement condition is shown alongside the corresponding structure for CHO line 1 (A) and CHO line 3 (B).

CONCLUSIONS

BD Resurge CD PAK, a diverse set of CD supplements/feeds, was developed using an iterative, DOE-based approach across multiple CHO cell lines. The formulations in the set are suitable for use in different cell culture systems. The development process for BD Resurge recognized that the performance of a CD supplement or feed varies with the culture conditions–the cell line, basal medium, supplement or feed, and other parameters of the application. The BD Resurge CD supplements were therefore developed as a kit, BD Resurge CD PAK, to provide a more comprehensive set of formulations for process development.

Although the CHO cell lines tested had different base media and process requirements, at least one CD supplement in the CD PAK provided benefit for growth, titer and/or viability. All the CD supplements in the CD PAK can be used both in batch and fed batch formats. For the cell lines tested, feeding of BD Resurge CD supplements resulted in increase in protein titer over batch cultures. The results reinforced the concept that each cell line requires a unique cell culture environment (base medium and supplement or feed) for optimal performance.

TRADEMARKS