Simplifying Progress

A Multi-Scale Approach To CHO Media Benchmarking

Claudia Kueppers, Michael Grauf, Tobias Schenk, Thomas Krieg, Dirk Mueller, and Christoph Zehe

Sartorius Stedim Cellca GmbH, D-89081 Ulm, Germany

Introduction

Competitive commercial CHO processes demand a balanced combination of media, process and production clone to achieve optimal performance. This study aimed was to apply a multi-scale approach to efficiently screen combinations of chemically-defined commercial media and various CHO production clones using benchtop bioreactor systems. Performance was assessed regarding cell growth, productivity, and concerning product N-glycosylation. The best clone media combinations were further characterized in 5L bioreactors and screening results were compared against a more traditional shake flask approach.

Methods

Four DG44 CHO clones, expressing IgG1 or IgG4 products, were cultivated in fed-batch mode, using commercial media and feed sets from five different suppliers. Before use in fed-batch, each clone was cultivated in the respective suppliers' stock culture medium for 4 weeks to ensure adaptation.

All clones and media were tested in shake flask mode at a culture volume of 25 mL and in parallel in the Ambr® 15 device at a culture volume of 14 mL.

Two selected clones and the two best-performing media were subsequently tested at 5L scale in a Univessel glass bioreactor. Product titers were determined using the ForteBio Octet Qke system and a LabChip GXII (PerkinElmer) was employed for analysis of product N-glycan profiles in final samples. Raw data was processed to compute integral viable cell density (IVCC) as a measure of cell growth.

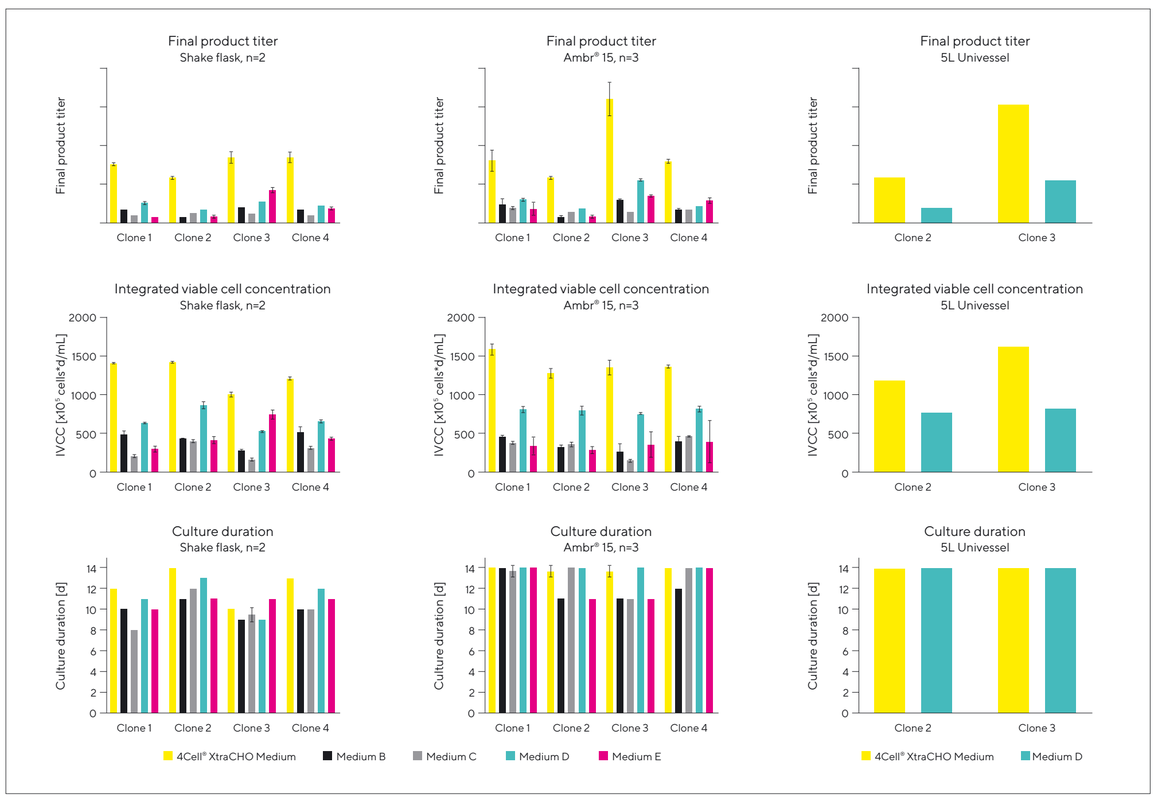

Figure 1: Product titers, integrated cell concentrations and culture duration in three cultivation systems, using 4 DG44 CHO cell clones and 5 sets of CHO culture media. Cultures were stopped on day 14 or when viability dropped below 70%.

Results

Titer and growth performance in 3 culture systems

In fed-batch screening, the performance was superior using Medium A for all tested clones with regard for product titer and cell growth across all culture systems (Figure 1). Medium D reproducibly performed second best, with the exception of Clone 3 in shake flasks.

For evaluation of media performance it needs to be considered that the maximum culture duration of 14 days was achieved more reliably in the controlled cultivation systems (Ambr® 15 and 5L bioreactor) than in shake flask format. For example, a good correspondence between the titers of Clone 3 in media A and D was observed in 5L and Ambr® 15 system, whereas the shake flask titer in both media was significantly lower - in line with the shorter process duration.

Moreover, Ambr® 15 cultures also correctly capture the improved growth (as judged by IVCC) of Clone 3 vs. Clone 2 at 5L scale – opposite to their respective growth in shake flask format. These findings illustrate that Ambr® 15 provides a better scaledown model for the present benchtop process than shake flask cultures and helps to reduce unintended bias in media evaluation.

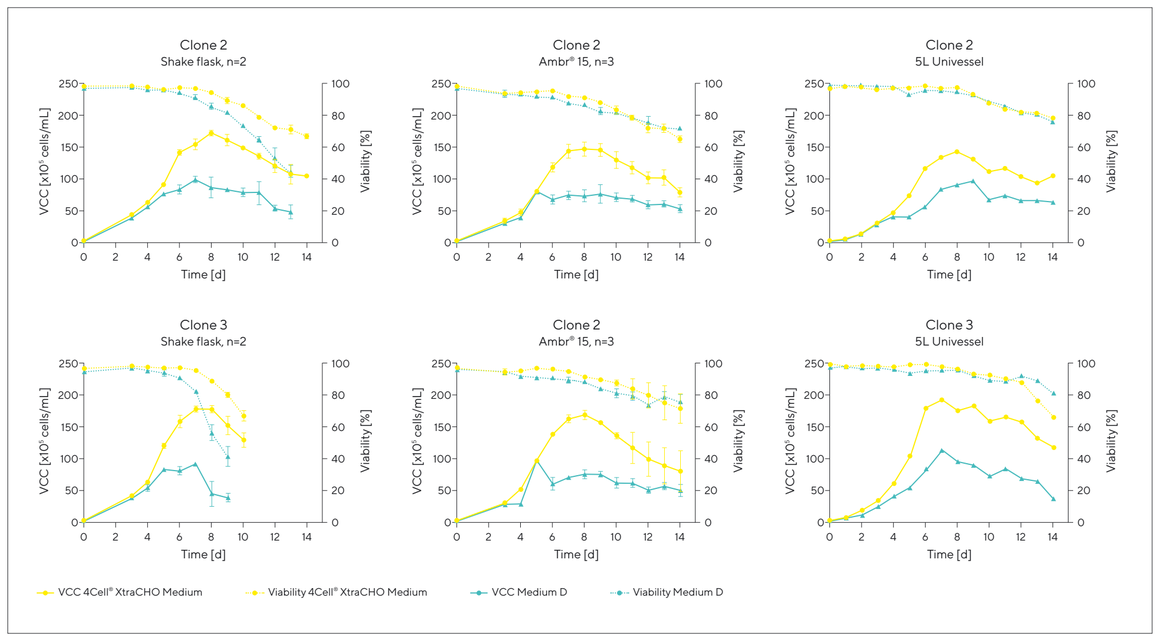

Figure 2: Profiles of viable cell concentration (VCC) and cell viability for two representative CHO clones in three cultivation systems for two of the culture media tested. Shake flask cultivations were performed in duplicate, Ambr® 15 runs in triplicate, and 5L cultivations as single runs. Error bars signify standard deviations where applicable.

Growth and viability profiles are illustrated for representative clone | media combinations in three culture systems (Figure 2). Differences between shake flask and Ambr® 15 cultivations were found to be minor for some clone/media setups (e.g. Clone 2 in Medium A); however, cell viability may drop below the harvest threshold one or several days earlier in shake flasks than in the controlled cultivation systems (Clone 2 in Medium D; Clone 3 in Medium A and D), leading to early termination of culture and lower product yield. This makes it difficult to reliably predict bioreactor performance from shake flask data alone and may lead to wrongly excluding certain media for a given clone (e.g. Medium E in lieu of Medium D for Clone 3) due to the application of a sub-optimal culture model.

Product N-glycosylation profiles show a high degree of comparability between Ambr® 15 and 5L bioreactor scales

Final day samples of Ambr® 15 and 5L bioreactor cultures were analyzed regarding product N-glycosylation profiles to assess the impact of different media and culture scales on product quality.

For all 4 proteins investigated, N-glycan distribution was product-specific, but significant and reproducible trends in glycan patterns were observed for all products when cultivating in different media (Figure 3). For example, cultures in Medium A show the smallest fraction of G0f in every product, while the same glycan is very high in Media D, E and F. G1'f, G1f and G2f are consistently highest in Medium A, followed by Media B and C, illustrating higher support of product galactosylation in the former.

For the two clones and two media investigated at 5L scale, glycosylation patterns were found to be highly similar to the respective Ambr® 15 cultures, underscoring the suitability of the controlled Ambr® 15 system for media screening purposes including product quality attributes.

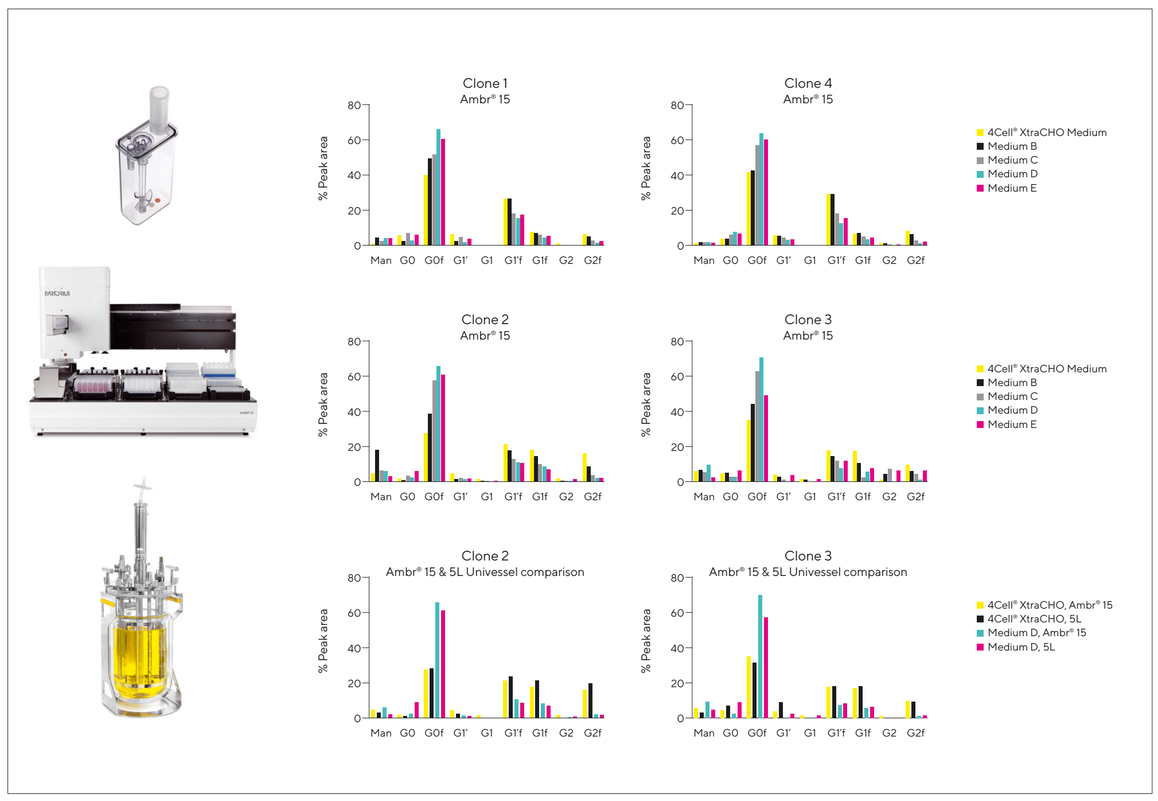

Figure 3: Product N-glycosylation profiles in final day samples of Ambr® 15 and 5L bioreactor cultures

Conclusions

- In Ambr® 15, most fed-batch cultures preserved high viability for several days longer than comparable shake flask cultures, enabling higher volumetric productivity and providing a better process model for 5L scale
- Ambr® 15 system provided a very good small-scale model for predicting N-glycosylation patterns at 5L stirred bioreactor scale
- N-glycosylation patterns of product proteins showed a clear and consistent trend for specific media across multiple product types → Underline the strong impact of media choice on product quality attributes and provide avenues for targeted adjustments