Development of DoE based fed-batch strategies for high-producing CHO cell cultures

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Development of DoE based fed-batch strategies for high-producing CHO cell cultures

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Abstract

Fed-batch culture is commonly employed to maximize cell and product concentrations in upstream mammalian cell culture processes. Typical standard platform processes rely on fixed-volume bolus feeding of concentrated supplements at regular intervals. However, such static approaches might result in over- or underfeeding. To mimic more closely the dynamics of a fed-batch culture, we developed a dynamic feeding strategy responsive to the actual nutrient needs of a mAb-producing recombinant Chinese hamster ovary (CHO) cell line.

Materials and methods

- Model cell line: mAb-expressing CHO DG44 cells (licensed from Cellca GmbH).
- Cultivation medium: HyClone™ CDM4NS0 medium (GE Healthcare) supplemented with glutamine.
- Feeds: HyClone Cell Boost™ 1, 2, 3, 4, 5, 6, 7a, and 7b supplements (GE Healthcare).
- Analytics: cell concentration, viability, mAb titer, selected metabolites, osmolality, and amino acids.
- Design of experiments (DoE) using MODDE™ statistical software (Umetrics AB) for development of fed-batch culture in three steps (Fig 1).

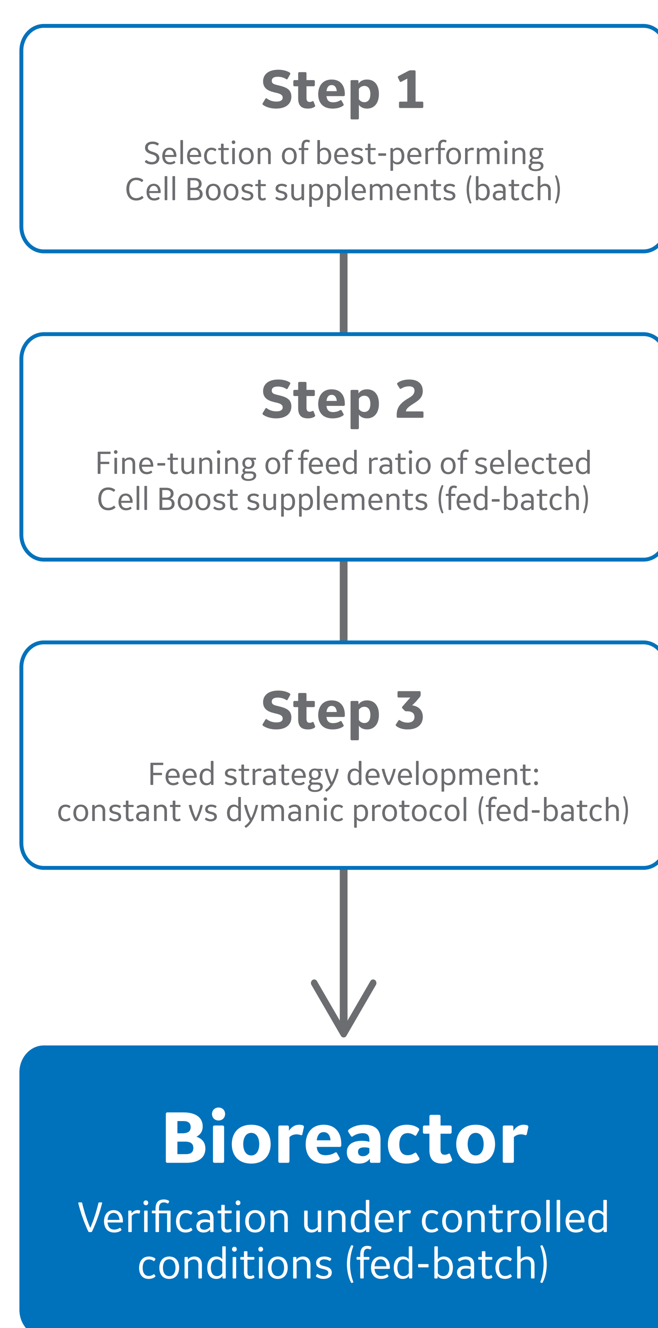

Fig 1. Strategy for development of fed-batch culture in three steps. The best results from Step 3 were verified under controlled conditions in automated bioreactor runs.

Conclusions

The established methodology for fed-batch culture development is a rapid protocol to select well-performing feed supplements and optimize their ratio to the culture requirements. In three steps, mAb titers were boosted 2.5× from 1.9 g/L to 4.9 g/L. Product glycosylation and charge variants could be influenced by the newly selected basal media and feeds compared with a legacy fed-batch process. The amount of aggregated product was not altered.

Results and discussion

Figure 2 gives an overview of the improvements made in different steps during development of a fed-batch culture process. At step 1, using a DoE approach in batch cultures, all eight Cell Boost supplements were added to CDM4NS0 medium. This evaluation allowed selection of only those Cell Boost supplements that were beneficial for the overall culture performance. Non-performing Cell Boost supplements were excluded from further studies.

At step 2, using a DoE approach in fed-batch cultures, the selected Cell Boost supplements were added daily to the cultures at different ratios. As expected, daily feed additions to replenish consumed nutrients substantially improved mAb and peak cell concentrations as well as viable cumulative cell days (VCCD) compared with batch cultivation. Further, the results enabled fine-tuning the feed ratio of selected Cell Boost supplements.

At step 3, the best-performing feeding approach was further optimized by investigation of static and dynamic feed protocols. Most fed-batch protocols rely on constant feed additions on distinct days. However, these approaches often lead to substantial over- or underfeeding of the culture. To optimize feeding of such "static" protocols, three different "dynamic" approaches, as shown in Table 1, were investigated by applying the selected Cell Boost supplements at optimized feed ratios. This investigation allowed further improving the bioprocess performance.

The best-performing approaches, constant and retrospective feed, were further investigated in automated bioreactor runs under controlled conditions. In general, constant bioreactor cultivation parameters slightly enhanced mAb titers compared with shake flask cultivation. The retrospective feed strategy yielded 10% higher titers than the constant strategy.

Overall, the established methodology for fed-batch culture development enabled 2.5× higher mAb titers (mean batch: 1.9 g/L vs mean fed-batch: 4.9 g/L) in three simple steps at short time. In addition, the product quality was investigated (Table 2). Compared with the legacy fed-batch process, fed-batch processes conducted with the newly selected basal media and feeds altered the distribution of charge and glycan variants. The amount of aggregated product was not altered.

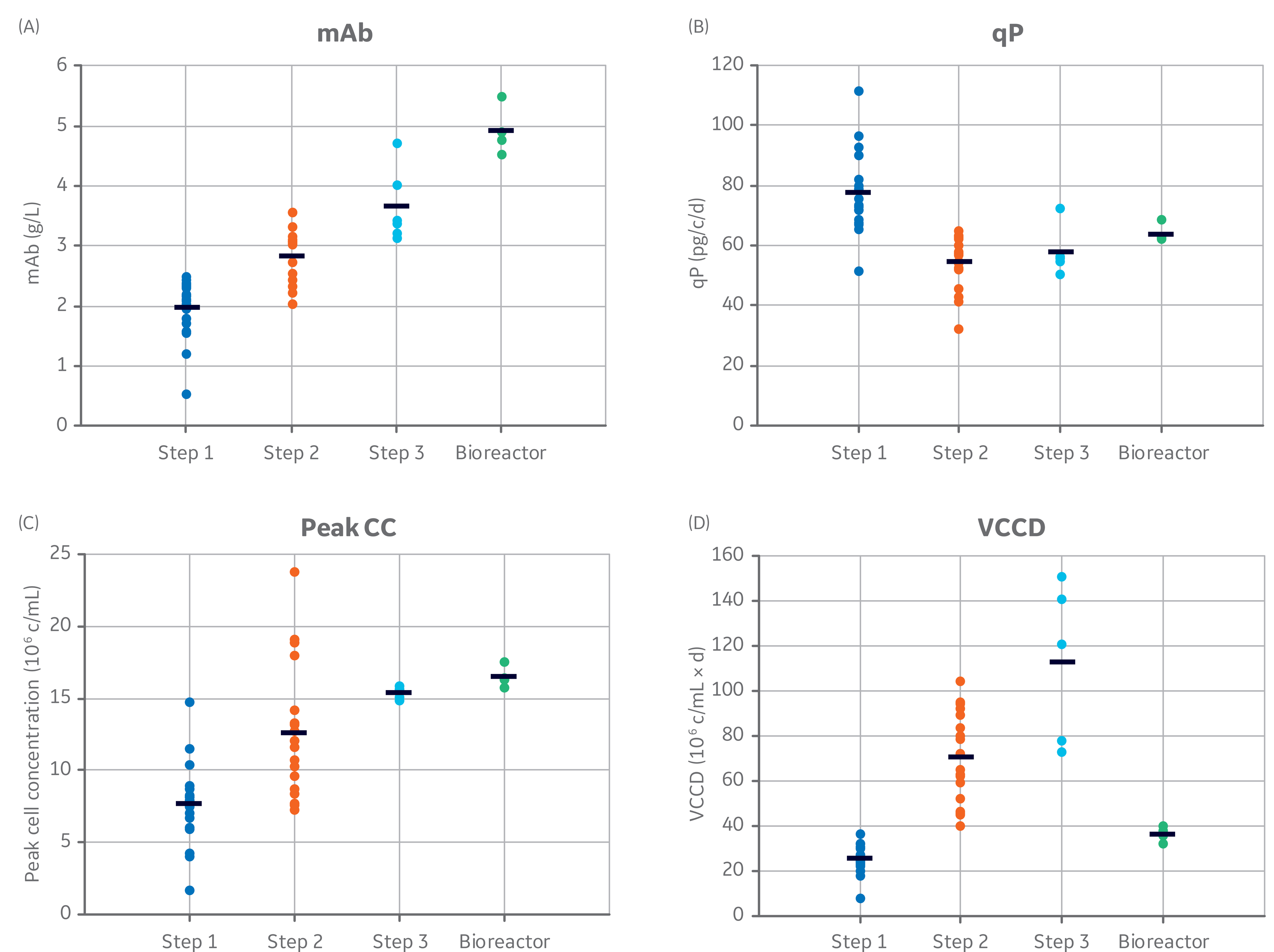

Fig 2. Overview of improvements in (A) mAb titer, (B) cell-specific mAb production rates (qP), (C) peak cell concentrations, and (D) the integral over the viable cell concentration (VCCD) at different steps during fed-batch culture development. Each experimental result is shown by a colored dot. Mean values of all experiments performed at each step are indicated by a black line.

Table 1. Strategies for static and dynamic fed-batch development

Fed-batch protocol	Fundamentals for daily feed additions
Static	Constant, daily feed additions.
Dynamic: concentration	Key substrate kept at target concentration.
Dynamic: predictive	Prediction of integrated viable cell count and cell-specific consumption rates of key substrate.
Dynamic: retrospective	Based on mean daily gains of viable cell concentration and mean cell-specific consumption rates of previous experiments.

Table 2. Product quality attributes

Analytical technology	Analyte	Legacy fed-batch process	CDM4NS0 process
CIEX	Acidic variants	60%	34% ± 2%
	Alkaline variants	3%	9% ± 0.2%
SEC	Aggregate	1%	1% ± 0.3%
Glycan map	G0F	35%	71% ± 2%
	G1F	41%	23% ± 2%
	G2F	15%	3.4% ± 0.2%
	Man5	3%	1.2% ± 0.1%

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