Optimization of fed-batch culture conditions for a mAb-producing CHO cell line

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This application note describes a broadly applicable and efficient workflow towards finding the optimal feed combination for best performing fed-batch processes. An IgG1-producing CHO cell line was cultured in HyClone™ CDM4NS0 medium, and individual HyClone Cell Boost™ feed supplements were screened in subsequent spiked batch and fed-batch experiments. Process development was supported by a design of experiment (DoE) approach to reduce the number of cultures required to infer valuable information on how certain feed combinations influence culture performance. In the first step, optimal feed combinations were screened by spiking the basal medium with different Cell Boost combination on day 0 of batch cultures. The selected Cell Boost supplements were thereafter applied in fed-batch cultures to fine-tune their relative ratios. The selected fed-batch culture conditions supported mAb titers of approximately 4 g/L in bioreactor cultures.

Introduction

Fed-batch processes are commonly applied in cell culture production to supply critical nutrients at physiological conditions and drive cell performance towards excellence. The best combination of high-performing basal medium and feed solution and the optimal feed regimen are often combined for a given cell line to boost antibody titers to g/L in straightforward fed-batch processes. In this application note, the workflow outlined in Figure 1 is exemplified for a mAb producing CHO cell line after medium screening and adaptation to CDM4NS0 basal medium (Step 0). After the initial basal medium screening, a DoE approach was used to identify the optimal combination of different Cell Boost solutions that enhanced cell culture performance in spiked batch experiments (Step 1). In this DoE study, levels ranged from no feed addition to a maximum addition for reaching a final osmolality of 400 mOsm/kg in the spiked CDM4NS0 medium. The optimal combination of Cell Boost supplements was thereafter applied in a fed-batch process by daily bolus addition of the feed solution (Step 2). Also at this stage, a DoE approach was used to was fine-tune the feed ratio added to the cell culture. In this second DoE study, the selected Cell Boost supplements were added to the basal medium to reach 400 to 600 mOsm/kg after ten simulated feed additions. In the two consecutive DoE experiments at small 30 mL scale, the best combination of Cell Boost supplements and their optimal amounts given as daily bolus feed additions were identified for the investigated fed-batch process. Optionally, the feeding strategy can be optimized by exploring different constant or dynamic feeding strategies based on cell culture demands (Step 3). This step, however, was excluded from this study. Finally, the optimal Cell Boost combination was used in a controlled bioreactor run at 500 mL scale, which showed similar or even slightly higher final product titers (Step 4).

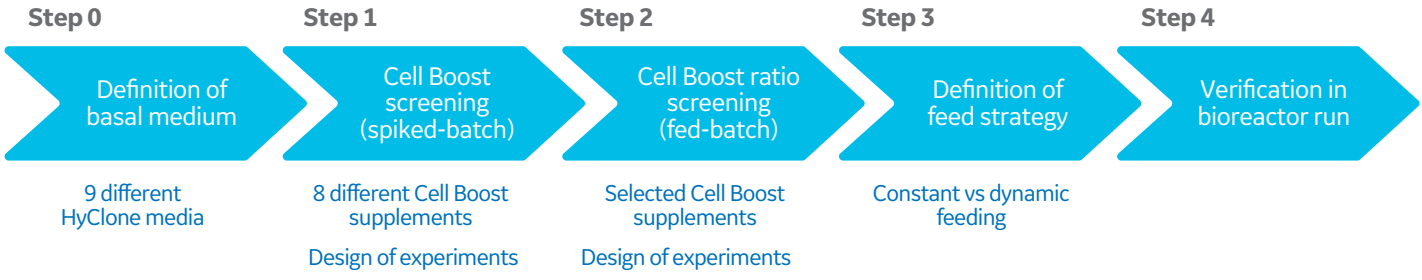


Fig 1. Proposed workflow towards a high-performing fed-batch process.

Materials and methods

Cell line and media

The CHO-DG44 cell line, expressing an IgG1 antibody directed against TNF-α, was used in all experiments. The CHO cells were adapted to CDM4NS0 basal medium (step 0). Eight different Cell Boost supplements (1, 2, 3, 4, 5, 6, 7a, 7b) were selected for supplementation of the chemically defined basal medium to drive culture performance to its peak maximum (Table 1). The supplements differ in their nutrient composition and concentration. Defining a molar ratio based on total amino acid content allows establishing a normalization factor for the individual supplements in Cell Boost mixtures. Cell Boost 1 was arbitrarily set as 100%.

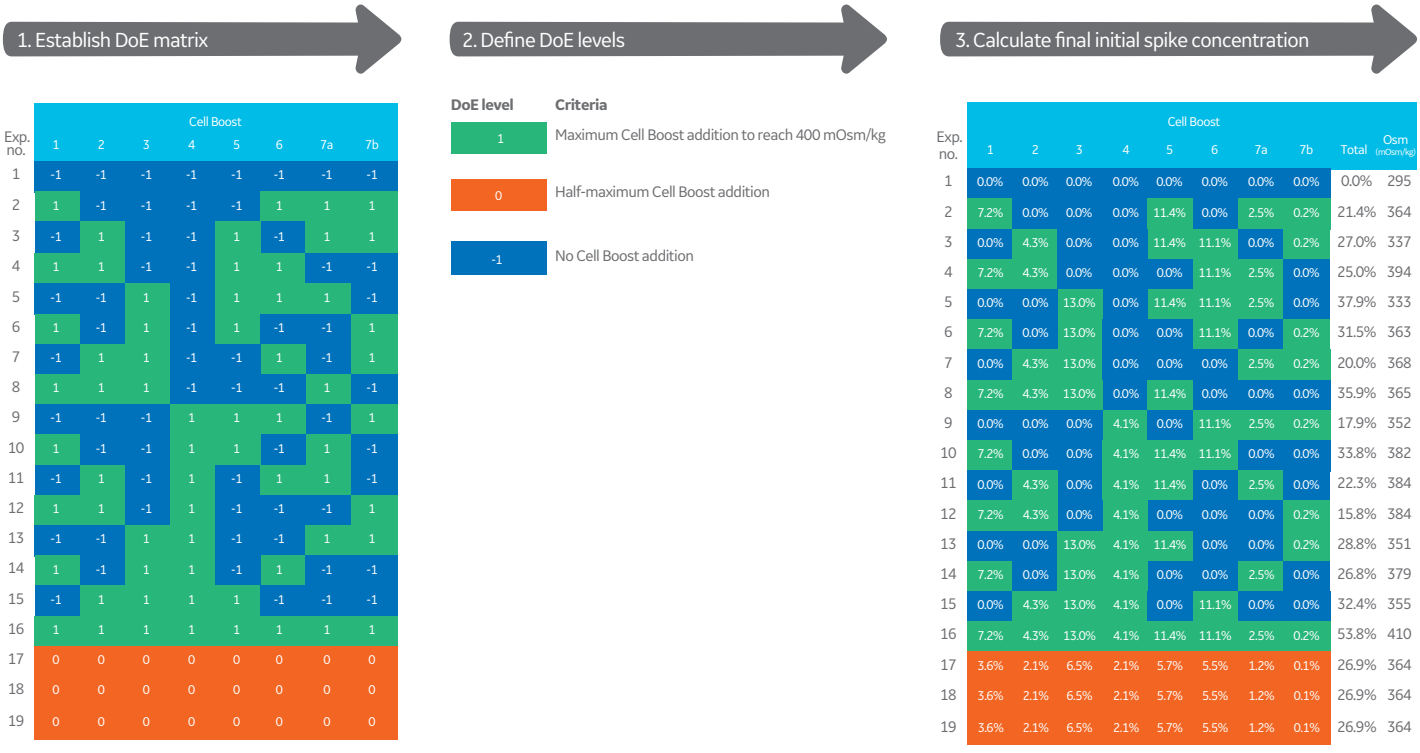
Table 1. Molar ratio of amino acids in stock solutions of Cell Boost supplements

Cell Boost supplement	Stock solution (%)	Molarity of total amino acids (mM)	Molar amino acid ratio
1	10	208.0	1.00
2	10	351.6	0.59
3	5	115.6	1.80
4	10	365.5	0.57
5	5	131.3	1.58
6	5	135.6	1.53
7a	18.1	603.0	0.34
7b	9.5	313.3	0.03

Note! For Cell Boost 7b, one tenth of the amount of Cell Boost 7a is ued.

Step 1. Screening of feed supplements

An optimal combination of Cell Boost supplements was empirically determined using a DoE approach (Fig 2). In the MODDE™ 12 software package (Umetrics AB), all eight Cell Boost supplements were entered as quantitative factors, using low, middle, and high DoE levels specified as -1, 0, and +1, respectively. Using the software's ***Design wizard***, a balanced subset of the full factorial design at two levels was recommended as a first choice, using three center points and a linear model. By this approach, an experimental plan using various combinations of different supplements spiked at different concentrations (i.e., DoE levels) to the basal medium was defined. This experimental plan typically contained controls such as basal medium without any Cell Boost supplementation (i.e., DoE level -1 in experiment no. 1), all Cell Boost supplements spiked at maximum concentrations (i.e., DoE level 1 in experiment no. 16), and all Cell Boost supplements spiked at half-maximum concentrations (i.e., DoE level 0 in experiment no. 17–19) as triplicate cultures. As a next step, the different DoE levels (-1, 0, and +1) were defined. The total amino acid concentration of each Cell Boost feed was used as a normalization factor. By mixing all Cell Boost supplements according to their molar ratio of total amino acid content (Table 1), each supplement contributes with the same total amino acid concentration (i.e., 10 mM) when this mix is spiked into the basal medium. According to Table 1, 1 mL of Cell Boost 1 was mixed with 0.59 mL, 1.8 mL, 0.57 mL, 1.58 mL, 1.53 mL, 0.34 mL, and 0.03 mL of Cell Boost 2, 3, 4, 5, 6, 7a, and 7b, respectively, to prepare an "all Cell Boost feed mix". This mix of balanced Cell Boost feeds was thereafter spiked into the basal CDM4NS0 medium to reach a maximum osmolality of 400 mOsm/kg to define the



Exp. no.	1	2	3	4	5	6	7a	7b
1	-1	-1	-1	-1	-1	-1	-1	-1
2	1	-1	-1	-1	-1	1	1	1
3	-1	1	-1	-1	1	-1	1	1
4	1	1	-1	-1	1	1	-1	-1
5	-1	-1	1	-1	1	1	1	-1
6	1	-1	1	-1	1	-1	-1	1
7	-1	1	1	-1	-1	1	-1	1
8	1	1	1	-1	-1	-1	1	-1
9	-1	-1	-1	1	1	1	-1	1
10	1	-1	-1	1	1	-1	1	-1
11	-1	1	-1	1	-1	1	1	-1
12	1	1	-1	1	-1	-1	-1	1
13	-1	-1	1	1	-1	-1	1	1
14	1	-1	1	1	-1	1	-1	-1
15	-1	1	1	1	1	-1	-1	-1
16	1	1	1	1	1	1	1	1
17	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0

Exp. no.	1	2	3	4	5	6	7a	7b	Total	Osm (mOsm/kg)
1	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	295
2	7.2%	0.0%	0.0%	0.0%	11.4%	0.0%	2.5%	0.2%	21.4%	364
3	0.0%	4.3%	0.0%	0.0%	11.4%	11.1%	0.0%	0.2%	27.0%	337
4	7.2%	4.3%	0.0%	0.0%	0.0%	11.1%	2.5%	0.0%	25.0%	394
5	0.0%	0.0%	13.0%	0.0%	11.4%	11.1%	2.5%	0.0%	37.9%	333
6	7.2%	0.0%	13.0%	0.0%	0.0%	11.1%	0.0%	0.2%	31.5%	363
7	0.0%	4.3%	13.0%	0.0%	0.0%	0.0%	2.5%	0.2%	20.0%	368
8	7.2%	4.3%	13.0%	0.0%	11.4%	0.0%	0.0%	0.0%	35.9%	365
9	0.0%	0.0%	0.0%	4.1%	0.0%	11.1%	2.5%	0.2%	17.9%	352
10	7.2%	0.0%	0.0%	4.1%	11.4%	11.1%	0.0%	0.0%	33.8%	382
11	0.0%	4.3%	0.0%	4.1%	11.4%	0.0%	2.5%	0.0%	22.3%	384
12	7.2%	4.3%	0.0%	4.1%	0.0%	0.0%	0.0%	0.2%	15.8%	384
13	0.0%	0.0%	13.0%	4.1%	11.4%	0.0%	0.0%	0.2%	28.8%	351
14	7.2%	0.0%	13.0%	4.1%	0.0%	0.0%	2.5%	0.0%	26.8%	379
15	0.0%	4.3%	13.0%	4.1%	0.0%	11.1%	0.0%	0.0%	32.4%	355
16	7.2%	4.3%	13.0%	4.1%	11.4%	11.1%	2.5%	0.2%	53.8%	410
17	3.6%	2.1%	6.5%	2.1%	5.7%	5.5%	1.2%	0.1%	26.9%	364
18	3.6%	2.1%	6.5%	2.1%	5.7%	5.5%	1.2%	0.1%	26.9%	364
19	3.6%	2.1%	6.5%	2.1%	5.7%	5.5%	1.2%	0.1%	26.9%	364

Fig 2. Experimental design matrix and definition of DoE levels for Cell Boost spiked batch cultures of the mAb-producing CHO cell line grown in CDM4NS0 medium (Step 1). Each Cell Boost supplement contributes with 10 mM total amino acids.

maximum DoE level +1. A high osmolality above 400 mOsm/kg is often considered as a critical upper limit in spiked media, above which cell performance is detrimentally impacted. The lowest DoE level -1 is defined as no Cell Boost addition and DoE level 0 as the half-maximum Cell Boost addition. Using this approach, the "all Cell Boost mix" was added to CDM4NS0 at 0%, 26.89%, and 53.78% for DoE levels -1, 0, and +1, respectively, resulting in final osmolalities of 295, 364, and 410 mOsm/kg, respectively. Thereby, each Cell Boost feed additionally provided 0, 7.5, or 15 mM of total amino acids to the basal medium at level -1, 0, and +1, respectively.

The different Cell Boost spiked CDM4NS0 media were thereafter used for simple batch cultivations, using the investigated mAb-producing cell line at a starting cell concentration of 0.3 × 10^6 c/mL as 30 mL cultures in CDM4NS0 supplemented with 6 mM L-glutamine in 50 mL shake tubes at 220 rpm, 37°C, 7% CO_2, and 90% relative humidity. Sampling of cell concentration, viability, antibody concentration, and metabolites (i.e., glucose, lactate, glutamine, glutamate, and ammonium) was performed directly after inoculation on day 3, and then every day onwards. The cultures were terminated when the viability dropped below 60%.

Step 2. Defining ration of feed supplements

The optimal Cell Boost combination identified in step 1 was used in fed-batch culture using another DoE matrix (Fig 3). In this experimental plan, Cell Boost 3 was kept at a constant level of 6.5% of the working volume, as this feed showed good performance when added to the basal medium. The established experimental plan consisted of a total of 16 tubes. Additionally, DoE levels were extended to 1.5 for selected conditions. As fed-batch culture was applied in Step 2, the different DoE levels -1, 0, +1, and +1.5 were defined using a different approach compared with Step 1. Only the selected Cell Boost 1, 2, 3, 7a, and 7b were now mixed together according to their total amino acid ratio. It was observed that Cell Boost 7b might form precipitates when mixed with other Cell Boost supplements. Hence, Cell Boost 7b was always added separately to the culture. Selected Cell Boost supplements were spiked into CDM4NS0 to reach 400, 500, and 600 mOsm/kg for DoE level -1, 0 and +1, respectively. It was assumed that this would be the final osmolality after ten theoretical feed days. Therefore, the total amount of Cell Boost supplements to reach 400, 500, or 600 mOsm/kg was divided by ten to obtain the final daily feed addition for each supplement at a certain DoE level.

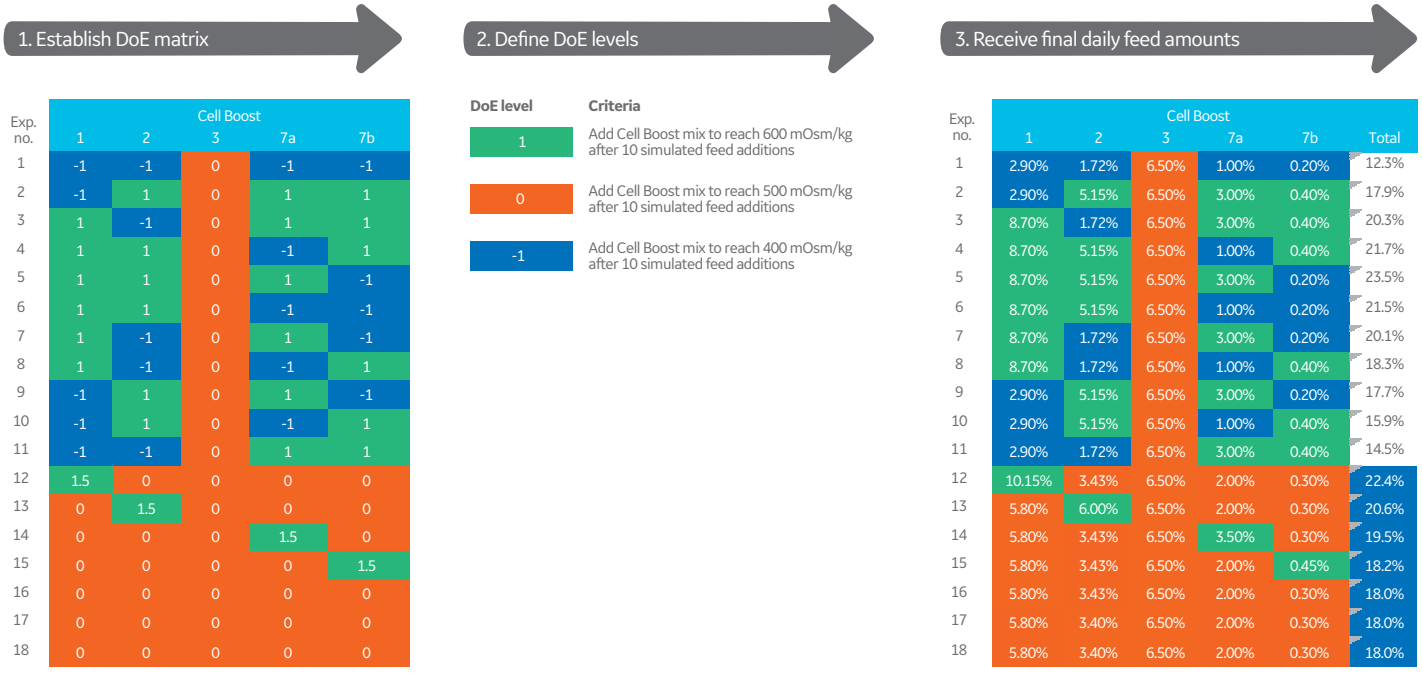
Cultures were seeded at 0.3 × 10^6 c/mL in CDM4NS0 supplemented with 6 mM L-glutamine in 50 mL shaking tubes at a working volume of 25 mL at 220 rpm, 37°C, 7% CO_2, and 90% relative humidity. Starting on day 3, feeds were added once daily according to Figure 3.

Step 4. Process verification in bioreactor runs

The mAb5 cell line in CDM4NS0 basal medium was cultured in a 0.5 L DASGIP (Eppendorf) fed-batch fermentation run to verify the developed Cell Boost mix and feeding strategy (Table 2).

Table 2. Different formulations of the optimized feed solution and daily concentration based on the current working volume (WV)

Feed solution	Included supplements	Bolus feed addition	Comment
Reference feed	Cell Boost 1, 2, 3, 7a, 7b	8.77% of WV	Cell Boost supplements prepared individually and mixed as liquids.
Feed mix	Cell Boost 1, 2, 3, 7a	8.70% of WV	Cell Boost 7b added separately at 0.07% of WV.



Exp. no.	Cell Boost 1	2	3	7a	7b
1	-1	-1	0	-1	-1
2	-1	1	0	1	1
3	1	-1	0	1	1
4	1	1	0	-1	1
5	1	1	0	1	-1
6	1	1	0	-1	-1
7	1	-1	0	1	-1
8	1	-1	0	-1	1
9	-1	1	0	1	-1
10	-1	1	0	-1	1
11	-1	-1	0	1	1
12	1.5	0	0	0	0
13	0	1.5	0	0	0
14	0	0	0	1.5	0
15	0	0	0	0	1.5
16	0	0	0	0	0
17	0	0	0	0	0
18	0	0	0	0	0

Exp. no.	Cell Boost 1	2	3	7a	7b	Total
1	2.90%	1.72%	6.50%	1.00%	0.20%	12.3%
2	2.90%	5.15%	6.50%	3.00%	0.40%	17.9%
3	8.70%	1.72%	6.50%	3.00%	0.40%	20.3%
4	8.70%	5.15%	6.50%	1.00%	0.40%	21.7%
5	8.70%	5.15%	6.50%	3.00%	0.20%	23.5%
6	8.70%	5.15%	6.50%	1.00%	0.20%	21.5%
7	8.70%	1.72%	6.50%	3.00%	0.20%	20.1%
8	8.70%	1.72%	6.50%	1.00%	0.40%	18.3%
9	2.90%	5.15%	6.50%	3.00%	0.20%	17.7%
10	2.90%	5.15%	6.50%	1.00%	0.40%	15.9%
11	2.90%	1.72%	6.50%	3.00%	0.40%	14.5%
12	10.15%	3.43%	6.50%	2.00%	0.30%	22.4%
13	5.80%	6.00%	6.50%	2.00%	0.30%	20.6%
14	5.80%	3.43%	6.50%	3.50%	0.30%	19.5%
15	5.80%	3.43%	6.50%	2.00%	0.45%	18.2%
16	5.80%	3.43%	6.50%	2.00%	0.30%	18.0%
17	5.80%	3.40%	6.50%	2.00%	0.30%	18.0%
18	5.80%	3.40%	6.50%	2.00%	0.30%	18.0%

Fig 3. Experimental design matrix and definition of DoE levels for fed-batch cultures of the mAb-producing CHO cell line grown in CDM4NS0 medium (Step 2).

The bioreactor was filled with 0.5 L of CDM4NS0 basal medium. After equilibration to pH 7.0 and 37°C, viable cells were seeded at 0.3 × 10^6 c/mL. Dissolved oxygen (DO) was maintained above 30% by adjusting air flow and oxygen concentration and the suspension was agitated constantly at 80 rpm. Starting on day 3, the culture was fed once daily with a constant 8.77% feed amount using different feed solutions prepared from powder or liquid Cell Boost formulations. For Feed mix 2, Cell Boost 7b was added separately into the bioreactor, using an additional feed tubing line. Critical metabolites (glucose, lactate, L-glutamine, glutamate, and ammonia) were measured daily. Cultures were harvested once the viability reached 70%.

Results and discussion

Screening of Cell Boost supplements in spiked batches (Step 1)

Using a DoE approach, different combinations of Cell Boost supplements were spiked in CDM4NS0 basal medium in batch cultures. For the best-performing combinations, peak cell concentrations could be increased from 8 × 10^6 c/mL of the unspiked control up to 15 × 10^6 c/mL (Fig 4). The integral of viable cells increased from 23 × 10^6 c × d/mL to more than 37 × 10^6 c × d/mL, resulting in a 2.6-fold increase in final mAb titer from 1.2 g/L up to 2.5 g/L on day 9.

In-depth analysis of the DoE experiment by regression analysis and model generation indicated a combination of Cell Boost 1, 2, 3, 7a, and 7b to be optimal to enhance batch performance of the investigated cell line grown in CDM4NS0 medium. Cell Boost 3 was shown to have a beneficial effect on cell growth and productivity, whereas Cell Boost 5 and 6 showed low performance and were therefore excluded from further studies.

Optimization of Cell Boost ratios in fed-batch cultures (Step 2)

The selected combination of Cell Boost 1, 2, 3, 7a, and 7b from Step 1 was further applied in a fed-batch process (Fig 5). Using a DoE approach, the Cell Boost combination was fed to the cultures to find the optimal daily feed ratio of each Cell Boost solution. Peak cell concentrations ranged from 8 × 10^6 to 24 × 10^6 c/mL, reaching final titers of up to 3.5 g/L.

The broad spectrum of experimental conditions allows to empirically find Cell Boost combinations at optimal ratios. However, as the experiment was designed according to a DoE approach, models can also be established using the MODDE software, allowing calculation of optimized conditions that cannot be identified empirically. As a final output, the DoE approach suggested a daily optimal feed ratio of Cell Boost 1, 2, 3, 7a, and 7b at 1.59%, 2.37%, 3.44%, 1.30%, and 0.07%, respectively, of the current working volume for the mAb-producing cell line grown in CDM4NS0 medium in a fed-batch process. To lower osmolality of the final feed solution, Cell Boost 7b can be excluded from the feed combination.

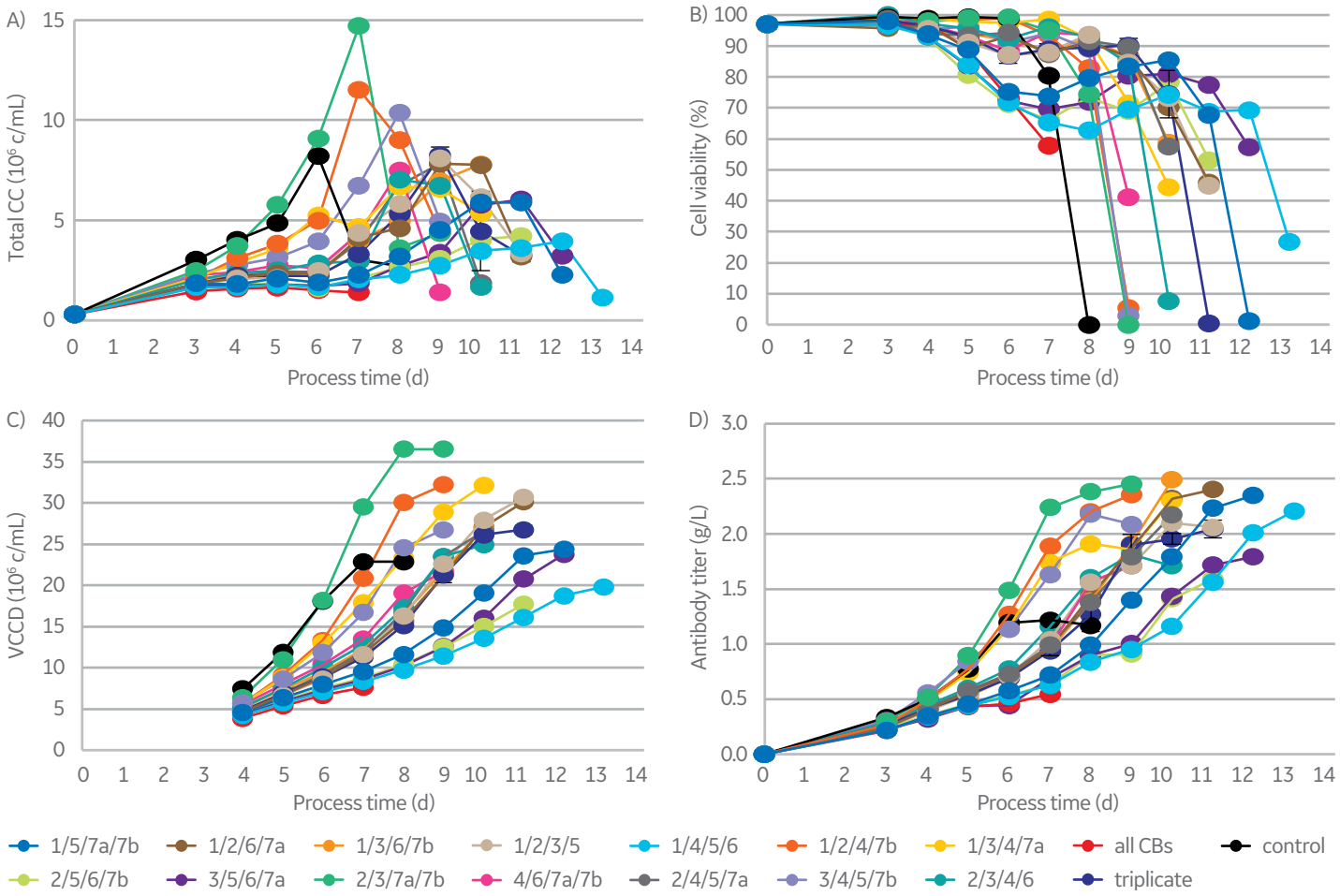


Fig 4. Cell growth and productivity for Step 1. (A) Total cell concentrations (CC), (B) cell viability, (C) viable cumulative cell days (VCCD), and (D) mAb titer.

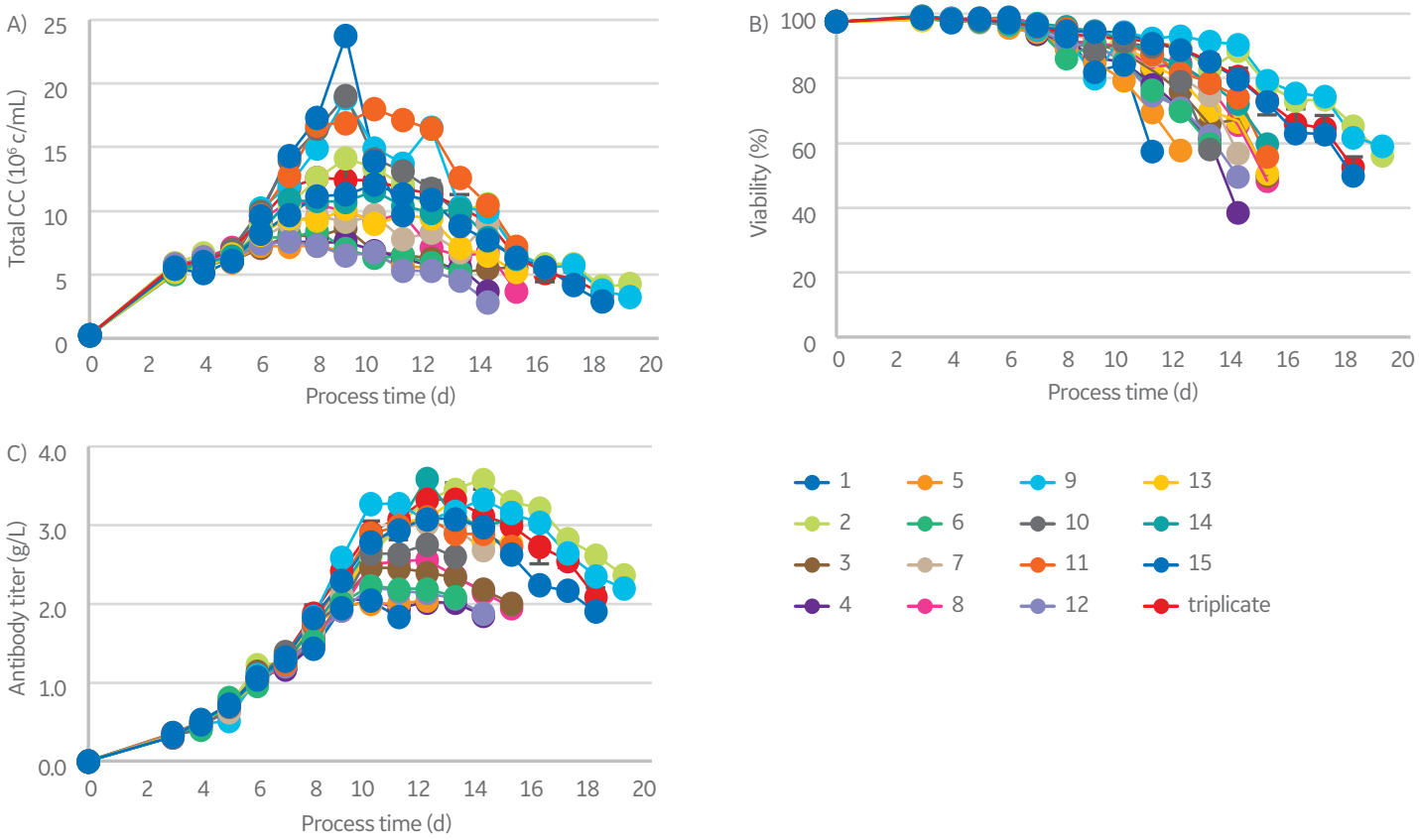


Fig 5. Cell growth and productivity for Step 2. (A) Total cell concentrations (CC), (B) cell viability, and (C) mAb titer.

Process verification in bioreactor cultures (Step 4)

Figure 6 gives a summary of cell culture data for the two DoE screening steps (Steps 1 and 2) and the final controlled bioreactor run (Step 4), in which the optimized feed composition and daily constant 8.77% feed regimen was verified. The same Cell Boost combination was prepared as different formulations and fed to the cultures according to Figure 7A. Feed solution was prepared from premixed powder formulations (Feed mix). It is recommended that Cell Boost 7b is added separately to the culture, as this supplement can form extensive precipitates when mixed with other Cell Boost feeds. As reference, the selected Cell Boost supplements were prepared individually and added separately (Reference feed).

Comparable peak cell concentrations of 23 × 10^6 c/mL were reached in both the reference culture and in the Feed mix culture to which Cell Boost 7b was added separately (Fig 7B). Similarly, maximum peak titers of 4 g/L were reached under both tested conditions after 10 days (Fig 7C). The nutrient composition was the same for both tested feed solutions. For both the reference culture and Feed mix, the osmolality remained relatively constant at 300 mOsm/kg over the entire fed-batch process (Fig 7D). The glycan distribution was comparable between the two runs (Fig 7E).

Fig 6. Summary of cell culture data for two DoE screening steps (Steps 1 and 2) and final bioreactor run (Step 4).

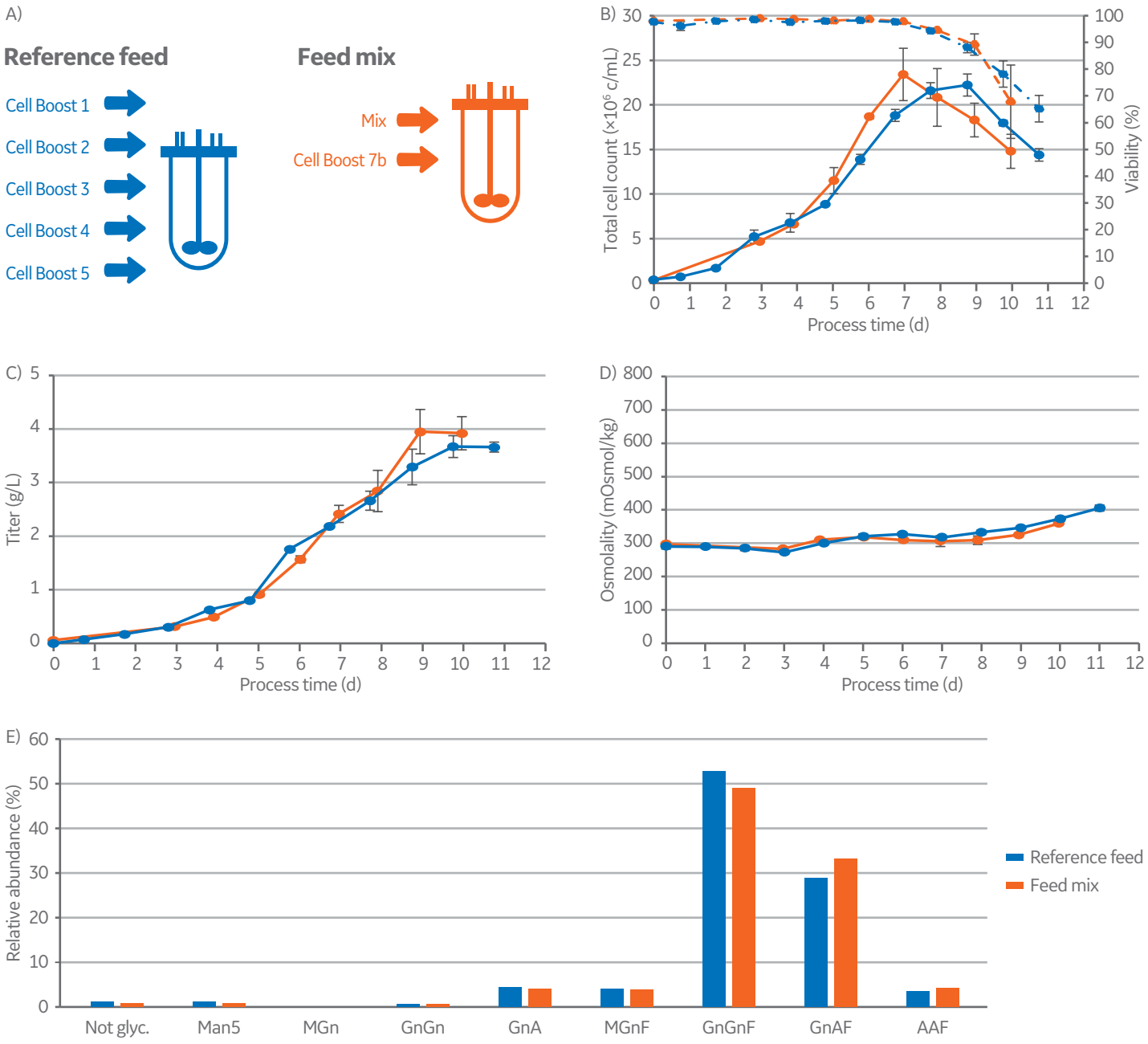


Fig 7. (A) Used feed solutions, (B) cell densities and viabilities, (C) mAb titers, and (D) osmolalities of the bioreactor cultures. Feed supplements were added at constant 8.77% daily feed regimen.

Conclusion

This work demonstrates a suitable workflow towards establishment of a high-performing fed-batch process that is also applicable to other cell culture medium and feed systems. Based on two consecutive DoE studies in batch and fed-batch mode, a combination of Cell Boost feed supplements was identified for maximum culture performance. For the investigated mAb-producing CHO cell line grown in CDM4NS0 medium, a constant 8.77% feed regimen, using a feed mix containing Cell Boost 1, 2, 3, 7a, and 7b (at 1.59%, 2.37%, 3.44%, 1.30%, and 0.07%, respectively), was found to drive antibody production beyond 4 g/L in a fed-batch process. Using the described workflow, a fed-batch process could rapidly be established that increased mAb production several-fold from unspiked batch culture thus simplifying optimization and increasing yield at the same time.

Ordering information

Product	Description	Product code
HyClone CDM4NS0	Basal medium	SH30579
HyClone Cell Boost 1	Feed supplement	SH30584
HyClone Cell Boost 2	Feed supplement	SH30596
HyClone Cell Boost 3	Feed supplement	SH30825
HyClone Cell Boost 7a	Feed supplement	SH31026
HyClone Cell Boost 7b	Feed supplement	SH31027

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