CHO cell perfusion culture development beyond 200 × 10^6 cells/mL

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Introduction

Perfusion culturing provides advantages like short product retention time and toxic byproduct removal. Here, we present a DoE-based workflow towards a CHO cell perfusion medium composition based on HyClone™ CDM4NS0 basal medium and Cell Boost™ supplements. TubeSpin bioreactors were used. The developed perfusion and basal media were verified using a ReadyToProcess WAVE™ 25 bioreactor system equipped with a 2 L Cellbag™ bioreactor. The developed process, run at 500 mL for 21 days, generated cell densities above 100 × 10^6 cells/mL with 90% viability for 12 days at 1–2 RV/d. Volumetric productivities between 1.5 and 6 g/L/d were achieved.

Materials and methods

Batch cultures

A recombinant IgG1 producing CHO-K1 cell line was seeded at 3 × 10^5 c/mL in basal HyClone CDM4NS0 medium supplemented with combinations of eight different HyClone Cell Boost feed supplements according to a first DoE matrix (Fig 1). For definition of DoE levels, all feeds, except Cell Boost 7b, were spiked in balanced amounts to contribute the same total amino acid content. Cell Boost 7b was added as 1/10^{th} of Cell Boost 7a volume. The maximum DoE level +1 was defined as a combination of all feeds to reach a final osmolality of 400 mOsm/kg when spiked into basal medium. DoE level -1 was defined as no feed addition and DoE 0 as half-maximum feed addition. DoE matrix design and establishment of final statistical models were performed using MODDE™ software. Concentrations of metabolites were measured with a BioProfile™ 100 Plus analyzer. Glucose was added to final 6 g/L if the threshold of 3 g/L was reached. Cultures were shaken at 220 rpm at 50 mm amplitude in a Kuhner shaker instrument at 7% CO_2 and 85% humidity at 37°C.

Spiked-batches

DoE 1 matrix

Exp. no.	CB 1	CB 2	CB 3	CB 4	CB 5	CB 6	CB 7a	CB 7b
– 1 (no CB)	•	•	•	•	•	•	•	•
2	•	•	•	•	•	•	•	•
3	•	•	•	•	•	•	•	•
4	•	•	•	•	•	•	•	•
5	•	•	•	•	•	•	•	•
6	•	•	•	•	•	•	•	•
7	•	•	•	•	•	•	•	•
– 8	•	•	•	•	•	•	•	•
9	•	•	•	•	•	•	•	•
10	•	•	•	•	•	•	•	•
11	•	•	•	•	•	•	•	•
– 12	•	•	•	•	•	•	•	•
– 13	•	•	•	•	•	•	•	•
14	•	•	•	•	•	•	•	•
15	•	•	•	•	•	•	•	•
– 16 (all CBs)	•	•	•	•	•	•	•	•
– 17–19 (triplicate)	•	•	•	•	•	•	•	•

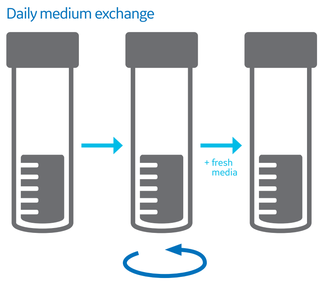
• -1 • 1 • 0

Fig 1. Cell boost screening (DoE 1).

Pseudo perfusion cultures

Cells were seeded at 10 × 10^6 c/mL in 10 mL basal medium spiked with different combinations of Cell Boost feeds preselected from previous batch experiments according to a second DoE matrix (Fig 2). DoE levels were defined the same as in the batch experiments. To simulate a perfusion culture at 1 RV/d, cells were centrifuged daily and re-seeded in fresh medium. For cell bleeding experiments, 20%, 30% or 40% of the cell suspension was removed before centrifugation.

Daily medium exchange



DoE 2 matrix

Exp. no.	CB 1	CB 2	CB 7a	CB 7b
– 1 (no CB)	•	•	•	•
2	•	•	•	•
3	•	•	•	•
4	•	•	•	•
5	•	•	•	•
6	•	•	•	•
7	•	•	•	•
8	•	•	•	•
9	•	•	•	•
10	•	•	•	•
11	•	•	•	•
– 12 (all CBs)	•	•	•	•
13	•	○	○	○
14	•	○	○	○
15	○	•	○	○
16	•	•	○	○
17	○	○	•	○
18	○	○	•	○
19	○	○	○	•
20	○	○	○	•
– 21–23 (triplicate)	○	○	○	○

• -1 • 1 • 0

Fig 2. Pseudo perfusion (DoE 2).

ReadyToProcess WAVE 25 bioreactor perfusion set-up

The optimized CDM4NS0 perfusion medium was applied in a steady state perfusion culture (Fig 3). Cells were seeded at 1 × 10^6 c/mL in unspiked CDM4NS0 basal medium. Perfusion was initiated on day 4 at 500 mL working volume and 1 RV/d perfusion rate. Once steady state was reached and maintained for five days, the perfusion rate was increased to 2 RV/d on day 13 or 14. Culture parameters were set to control > 30% DO, 37°C, 24 rpm at 9° angle, and pH 7.0.

Fig 3. ReadyToProcess WAVE 25 perfusion set-up.

Results

Eight different HyClone Cell Boost feeds were screened in batch experiments using a first design of experiment (DoE 1) to find optimal combinations to boost cell performance in CDM4NS0 medium. The peak cell concentrations varied from 3 × 10^6 c/mL (all Cell Boost feeds at level 1) to 26 × 10^6 c/mL (unspiked) and 37 × 10^6 c/mL (best combination), resulting in a final titer of more than 2.1 g/L antibody in a total process time of 14 days (Fig 4).

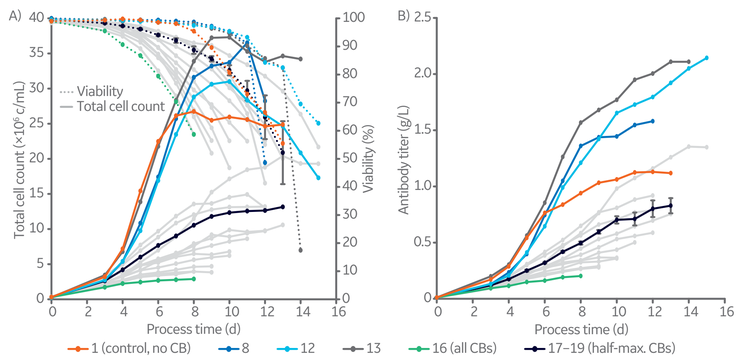


Fig 4. (A) Cell culture and (B) titer data from DoE 1. Cell Boost feeds were screened in batch experiments to find optimal combinations to improve culture performance in CDM4NS0 medium.

Based on the established DoE 1 matrix and generated experimental data, suitable models were established that recommended Cell Boost 1, 3, 7a, and 7b for further evaluation. In a second DoE approach, those preselected feeds were used to fine-tune the spike concentration into the basal medium for use in subsequent pseudo perfusion cultures by daily medium exchange.

Unspiked and glucose-spiked cultures showed a decline in viabilities after day 4 but high peak cell concentrations of 62 × 10^6 c/mL, reaching daily titers of 0.6 g/L at late culture time points. Supplementing basal medium with all feeds at maximum levels showed highest titers of up to 0.9 g/L, prolonged culture times, and high viabilities, but lower peak cell concentrations of around 50 × 10^6 c/mL (Fig 5). DoE analyses recommended an optimal spike concentration of 11.06% Cell Boost 1 and 19.90% Cell Boost 3 in CDM4NS0 as perfusion medium.

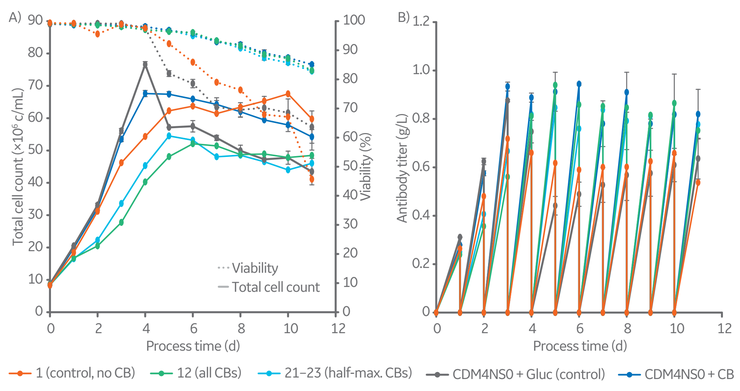


Fig 5. Cell culture data from DoE 2. In addition, data with the optimized medium (blue line) and CDM4NS0 spiked with glucose (17 g/L, grey line) were added to the data from the 23 experiments from the DoE.

The perfusion medium developed in the DoE strategy was also retrospectively tested in pseudo perfusion experiments with 20%, 30%, and 40% daily cell bleeds, which led to a successive reduction in peak cell densities from 62 × 10^6 c/mL down to 34 × 10^6 c/mL (Fig 6). Although daily cell bleeds reduced peak cell concentrations, the daily harvest titer could be increased from 0.9 g/L of the unbled cultures up to 1.0 g/L of the 20% or 30% bled cultures, indicating a correlation of cell-specific growth rate and productivities. Combining the developed perfusion medium and daily cell bleeds allowed a 1.8-fold titer increase.

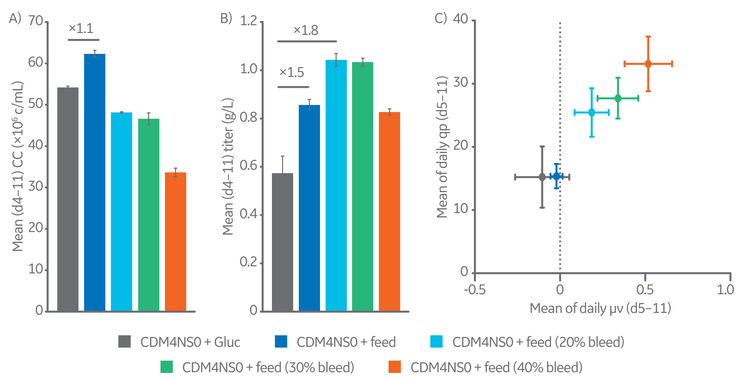


Fig 6. Effect of daily cell bleeding on growth and productivity.

The perfusion medium developed in the DoE strategy was used in a ReadyToProcess WAVE 25 bioreactor perfusion experiment. Peak cell densities of 140 × 10^6 c/mL were reached at a perfusion rate of 1 bioreactor volume per day (RV/d), which was boosted up to 225 × 10^6 c/mL at 2 RV/d with viabilities above 90% for a total of three weeks. This led to maximum perfusion titers of 1.4 g/L (1 RV/d) or 3.5 g/L (2 RV/d), which represents a significant improvement compared with 0.9 g/L (2 RV/d) of the basal medium spiked with glucose only. For all runs the cell-specific perfusion rate (CSPR) was below 13 pL/c/d at steady state (Fig 7). Because high cell densities were achieved during the feed spiked perfusion run, glucose became limiting, which forced the cultures to switch from lactate production to consumption (Fig 8). The proposed strategy for medium development using screening in pseudo perfusion cultures was shown to be suitable and predictable to develop high performing perfusion processes (Table 1).

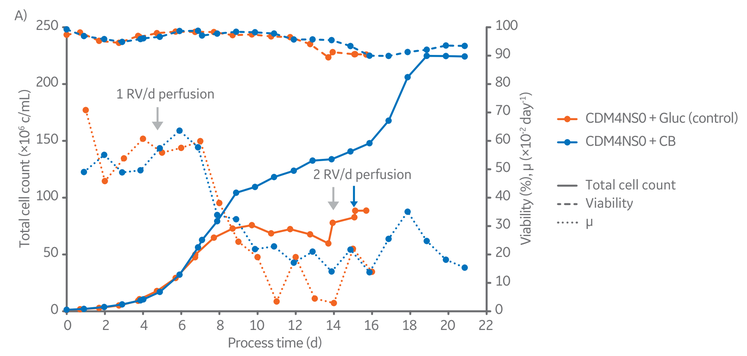


Fig 7. Cell culture data from ReadyToProcess WAVE 25 bioreactor perfusion experiment using CDM4NS0 medium fortified with either Cell Boost 1 and 3 (blue lines) or only corresponding glucose concentration (orange line).

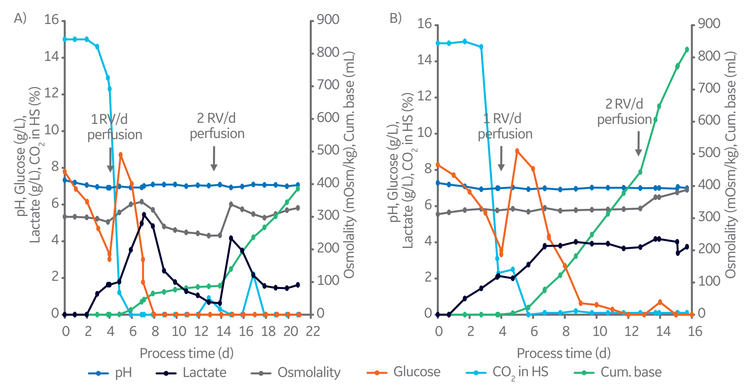


Fig 8. In-process data from ReadyToProcess WAVE 25 bioreactor perfusion experiment using CDM4NS0 medium fortified with either (A) Cell Boost 1 and 3 or (B) only corresponding glucose concentration.

Table 1. Process relevant data from pseudo-perfusion and ReadyToProcess WAVE 25 perfusion cultures

	CDM4NS0 + Gluc		CDM4NS0 + Cell Boost	
Mean (steady-state) values	**Pseudo perfusion**	**ReadyToProcess WAVE 25 (1 RV/d)**	**Pseudo perfusion**	**ReadyToProcess WAVE 25 (1 RV/d)**
CC (× 10^6 c/mL)	54	71	62	120
μ (day^{-1})	-0.1	0.1	0.0	0.2
Titer (g/L)	0.6	0.7	0.9	1.4
qP (pg/c/d)	14.8	9.7	15.4	12.8
Osmolality (mOsm/kg)	291	327	307	253
res. glucose (g/L)	6.1	0.3	4.0	0.0
Lactate (g/L)	3.0	3.9	2.6	1.3
res. glutamate (g/L)	0.20	0.05	0.20	0.06
qGluc consumption (pg/c/d)	255	220	220	148
qLac production (pg/c/d)	77	51	49	9
qGlu consumption (pg/c/d)	11	7	8	5

Conclusions

- A DoE-based screening of different feed supplements was used to identify a medium composition that was suitable for perfusion conditions in a bioreactor.
- This workflow illustrates that off-the-shelf media can serve as a convenient starting point for the rapid development of a candidate perfusion medium. The identified perfusion medium formulation can then be further optimized for manufacturability and stability.
- We demonstrated the feasibility of using simple batch and pseudo perfusion cultures as a streamlined model for perfusion media development.
- Pseudo perfusion cultures are highly suitable to investigate the effect of cell bleeding strategies.
- The identified medium from the screening was successfully transferred into a ReadyToProcess WAVE 25 bioreactor system to reach a maximum of 200 × 10^6 cells/mL at a volumetric productivity of 6 g/L/d and a low cell-specific perfusion rate of 6 pL/c/d.

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