From shake flask to SB10-X: Scaling a CHO fed-batch process

Consistent Scaling. Consistent Success.

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Eight 14-day CHO fed-batch cultivations from three bispecific antibody production campaigns were performed in the SB10-X (7 L starting volume, about 10 L final volume) and in shake flasks with identical medium and feed. The day-14 titer matched the shake flask (−4 %), and μ_{max} deviated by less than 7 %. Peak viable cell density was 12 % lower at the final process settings, mostly compensated by larger cells. Product quality differences were small (≤ 6 %) and not significant. Moderate O_2 enrichment of the headspace gas covered the oxygen demand while maintaining the volumetric power input.

Why 10 L orbital shaking?

Early development campaigns require antibody material in the double-digit gram range, more than shake flasks deliver and too little to justify the effort of a stirred-tank bioreactor and a process transfer. The SB10-X retains the flask principle (orbital shaking, surface aeration, no impeller) and adds DO and pH control, providing a simple route to early-stage material. This makes the SB10-X well suited for a fast and linear scale up from shake flask to a controlled cultivation at 10L scale.

Table 1. Process conditions (8 runs, 3 campaigns).

Parameter		
Cells	CHO-K1 pools/clones	
Temperature	37 °C → 32 °C (day 5)	
Feed	3.3 % working volume from day 3	
Inoculum	0.75 × 10^6 cells/mL	
Parameter	**SB10-X**	**Shake flask**
Volume	7 L C → 9.3–10.5 L final	50–100 mL start
Shaking	84–89 rpm, 50 mm	180 rpm, 50 mm
Headspace gas	200 mL/min*, O_2-enriched	5 % CO_2, 80 % rH
DO / pH	40 % / upper limit via CO_2	not controlled

* three early runs at 400–500 mL/min; commercial medium and feed

Results at a glance

Each SB10-X cultivation was compared with control flasks inoculated from the same seed culture and fed identically. Figure 1 shows an example run at the final process settings (reduced air flow, constant CO_2 concentration at the first three days). Figures 2 to 4 summarize all runs, averaged over eight cultivations.

−4 % titer day 14 (n.s.)	**−7 %** μ_{max} day 0–3	**−12 %** peak VCD at 200 mL/min
+13 % cell volume	**≤ 6 %** SEC and IEX profiles (n.s.)	**0.13** q_{O2} in pmol/(cell·h)

Deviation of the SB10-X from its control flasks; n.s., not significant (p >0.05).

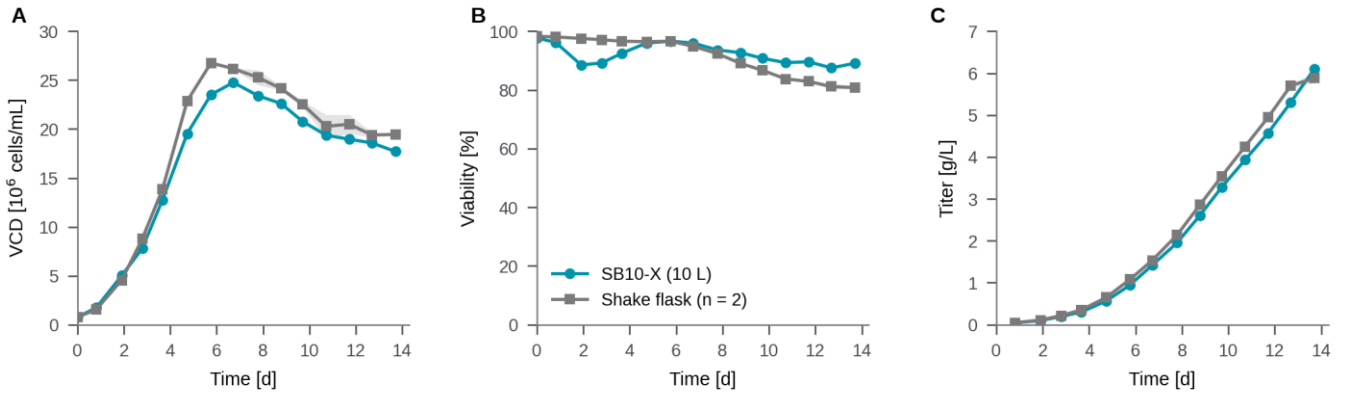


Figure 1. Example run at the final process settings. Viable cell density (A), viability (B) and titer (C) in the SB10-X and in two feed-identical control flasks (mean, range shaded). Data: CDR-Life AG.

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What scales and what not

Figure 2 compares each SB10-X cultivation with its own control flasks. The titer remained within the flask-to-flask variation, and μ_{max} deviated by less than 7 %. Peak viable cell density (VCD_{max}) was lower in all eight runs. However, the cells were 13 % larger, so the integral biovolume differed by only 10 %. The cell-specific productivity (q_P) increased accordingly, as described for the cell size increase phase of CHO fed-batch cultures [4].

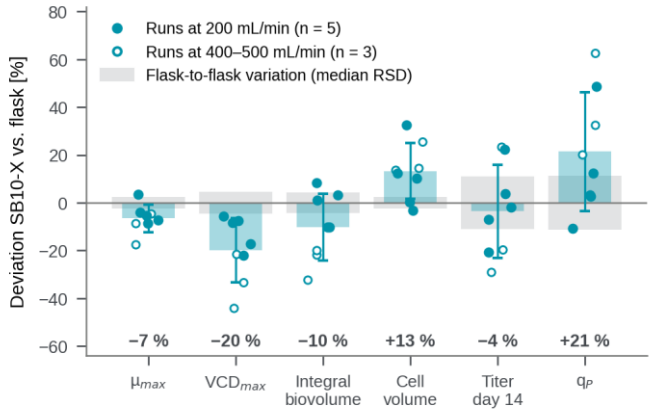


Figure 2. Deviation of the SB10-X from the control flasks of the same run (n = 8, bar = mean ± SD). μ_{max}, maximum specific growth rate (day 0–3); VCD_{max}, peak viable cell density; integral biovolume, time integral of VCD × cell volume; q_P, cell-specific productivity. Gray bars: flask-to-flask variation (median relative SD of the control flasks within a run).

The cell density gap depended on the headspace gas flow. In the three early runs at 400 to 500 mL/min, a third of the peak cell density was lost and the bag was partly operated at overpressure. At 200 mL/min, with the oxygen demand covered by further O_2 enrichment, the gap decreased to 12 %.

Oxygen supply

At the shaking frequency used (84–89 rpm), the volumetric mass transfer coefficient (k_La) of the SB10-X is about 12 1/h at 7 L and 8 1/h at 10 L (water, 37 °C) [1]. As the feed increases the volume, oxygen transfer decreases by a third (Fig. 3A).

The oxygen balance yields a cell-specific oxygen uptake rate (q_{O2}) of 0.13 pmol/(cell·h), about two thirds of the median reported for CHO cells [2–3]. At peak cell density, 20 to 60 % O_2 in the headspace gas was required, in line with the prediction from k_La and q_{O2} (Fig. 3B).

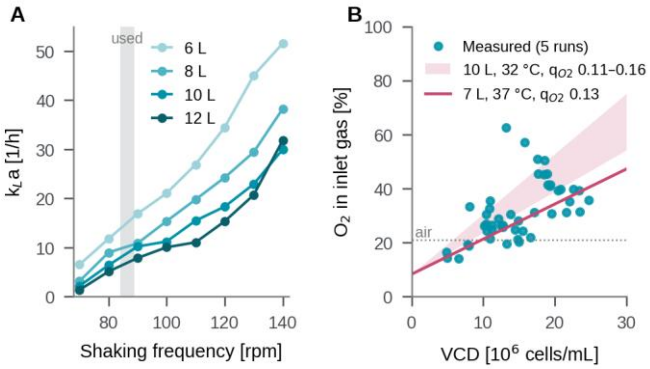


Figure 3. k_La of the SB10-X for 6–12 L (50 mm shaking diameter, water, 37 °C, 500 mL/min air); gray: shaking frequency used (A). O_2 fraction in the inlet gas at 200 mL/min, measured and predicted from the oxygen balance (B).

pH and CO_2

By design, no base was added. CO_2 in the headspace gas was the only pH actuator, and the pH set point therefore acted as an upper limit. The flasks were incubated under constant 5 % CO_2. pCO_2 in the SB10-X reached up to 15 kPa after day 8, i.e. after the cell density maximum, and did not explain the differences.

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Titer and product quality

The day-14 titer deviated by −4 % from the flasks (n.s., eight runs). SE-HPLC and IEX profiles of the purified harvest were compared with feed-identical control flasks of the same run (six runs). All differences remained within ±6 % and were not significant (Fig. 4). SEC main peak and HMWS stayed within the flask-to-flask variation. The IEX main peak was 6 % higher, with large differences between runs.

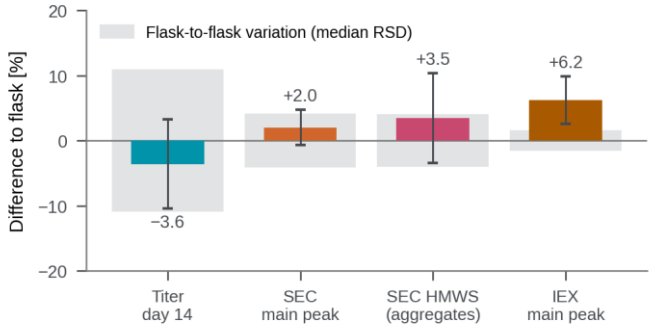


Figure 4. Relative deviation of the SB10-X from the control flasks: titer on day 14 (n = 8), SEC main peak, SEC high-molecular-weight species (HMWS) and IEX main peak (n = 6); mean ± SEM. Gray bars: flask-to-flask variation (median relative SD of the control flasks within a run).

Lessons learned

Headspace gas flow matters. High gas flow drove the bag into overpressure and coincided with the largest losses in cell density. Lower flow combined with O_2 enrichment performed better.

Biovolume complements cell counts. The cells grew larger in the SB10-X. Cell counts alone therefore overstate the difference.

The reference flask defines the comparison. A control flask with a different feed made a feed effect appear as a scale effect in product quality.

Recommendations

1. Define the flask reference first (same medium, feed relative to the start volume, inoculum density).

2. Plan the oxygen supply for the final volume and the peak cell density, using the oxygen uptake rate OUR = q_{O2} × VCD, since k_La decreases as the feed increases the volume.

3. Cover an increasing O_2 demand by enrichment rather than by higher headspace gas flow and monitor the bag pressure.

4. Record headspace gas flow, O_2 fraction and bag pressure together with the offline data.

5. Measure the cell diameter, compare biovolume and adjust cell-density-based criteria such as harvest or split points.

In summary

Across eight runs from three campaigns, the SB10-X at 10 L scale reproduced titer and growth rate of the shake flask, and product quality differences were not significant. Peak cell density was 12 % lower at the final gassing rate, with larger cells. If the oxygen supply is planned for the final volume and the headspace gas flow is kept moderate, a flask process can be transferred to 10 L without re-development.

References:

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