

TFDF®-Based Intensified Perfusion Cell Culture and Clarification for High-Productivity and Simplified Lentivirus Manufacturing

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Summary

The increasing demand for lentiviral vectors in chimeric antigen receptor T-cell (CAR-T) therapies necessitates more efficient manufacturing approaches.

This presentation demonstrates that implementing Tangential Flow Depth Filtration (TFDF®) technology for perfusion cell culture leads to a >10-fold increase in infective lentivirus (LV) yield compared to conventional batch processes. The continuous harvest of lentiviral particles, through the 2-5 micrometers pore size of the TFDF filter, not only enables high viral infectivity, but also streamlines the clarification process with throughput of secondary clarification filters >400 L/m², therefore reducing the filtration area requirements before the chromatography purification.

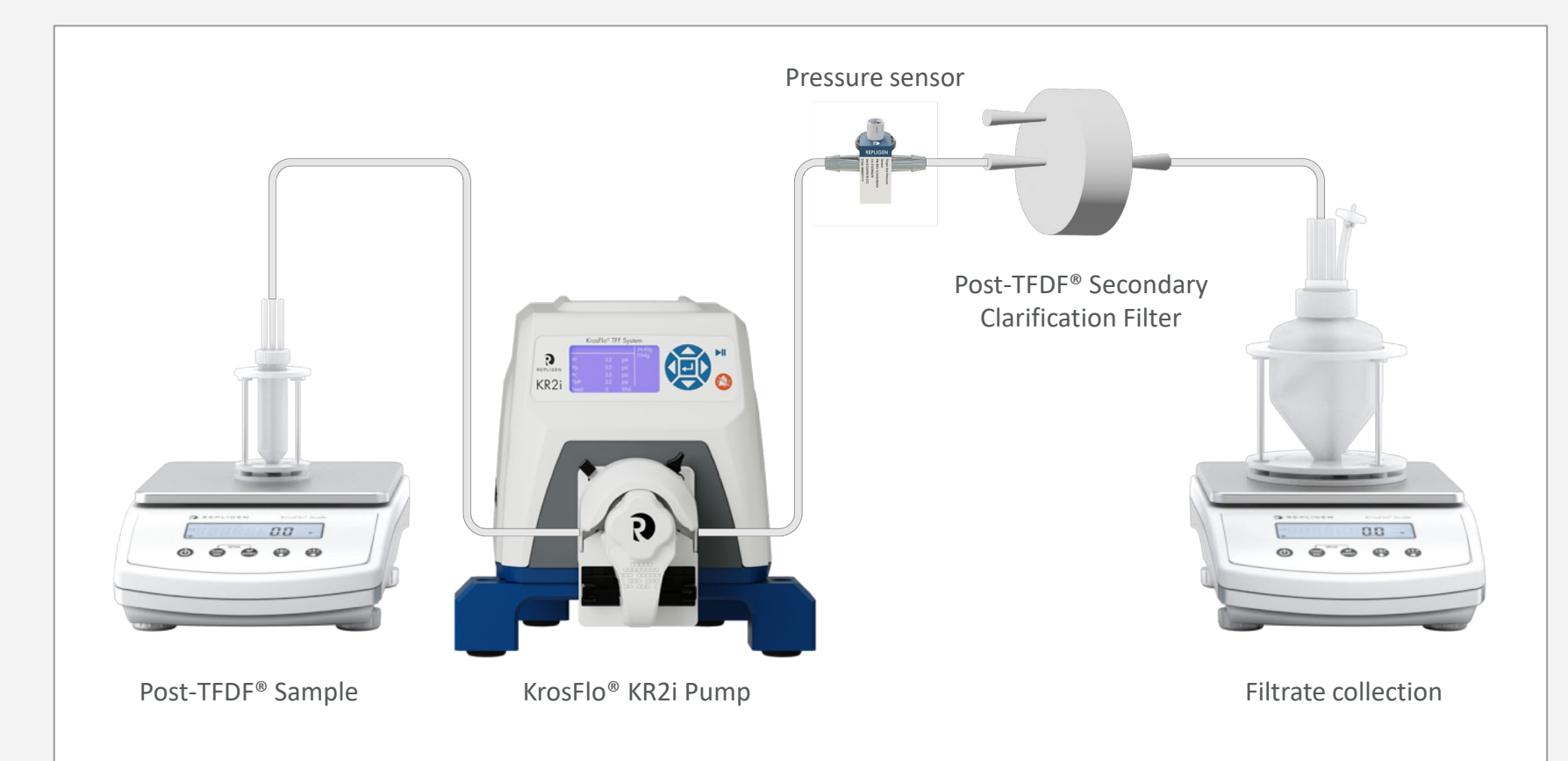
At 10 L bioreactor scale, the TFDF-intensified LV production process can generate sufficient doses for typical Phase II or III CAR-T clinical trials, leading to significant cost and footprint reductions, avoiding the need to scale up to 200 L batch bioreactor and using large and complicated harvest systems.

These advancements improve facility fit to accelerate the accessibility of CAR-T and other advanced therapies.

Materials and Methods

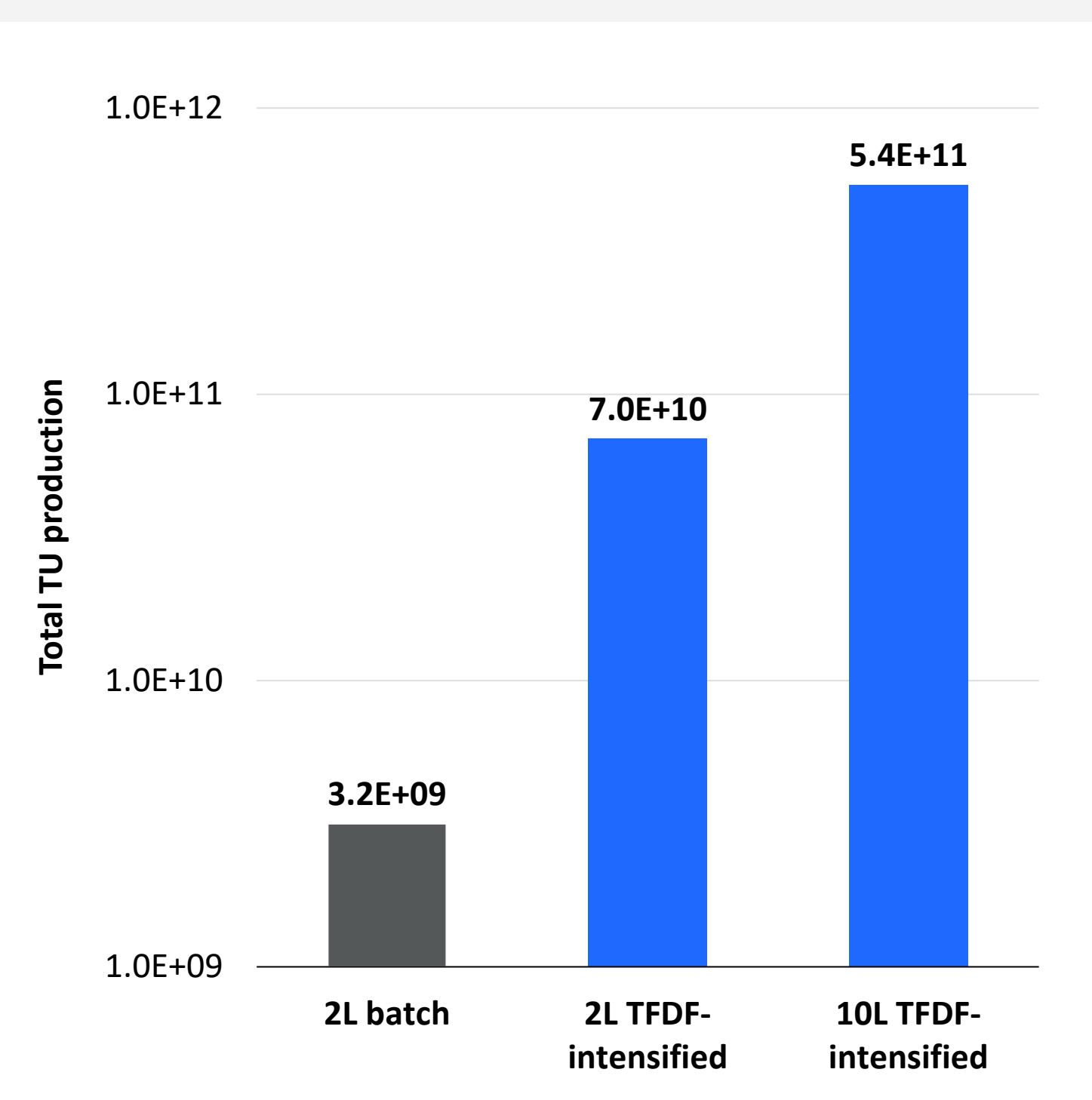
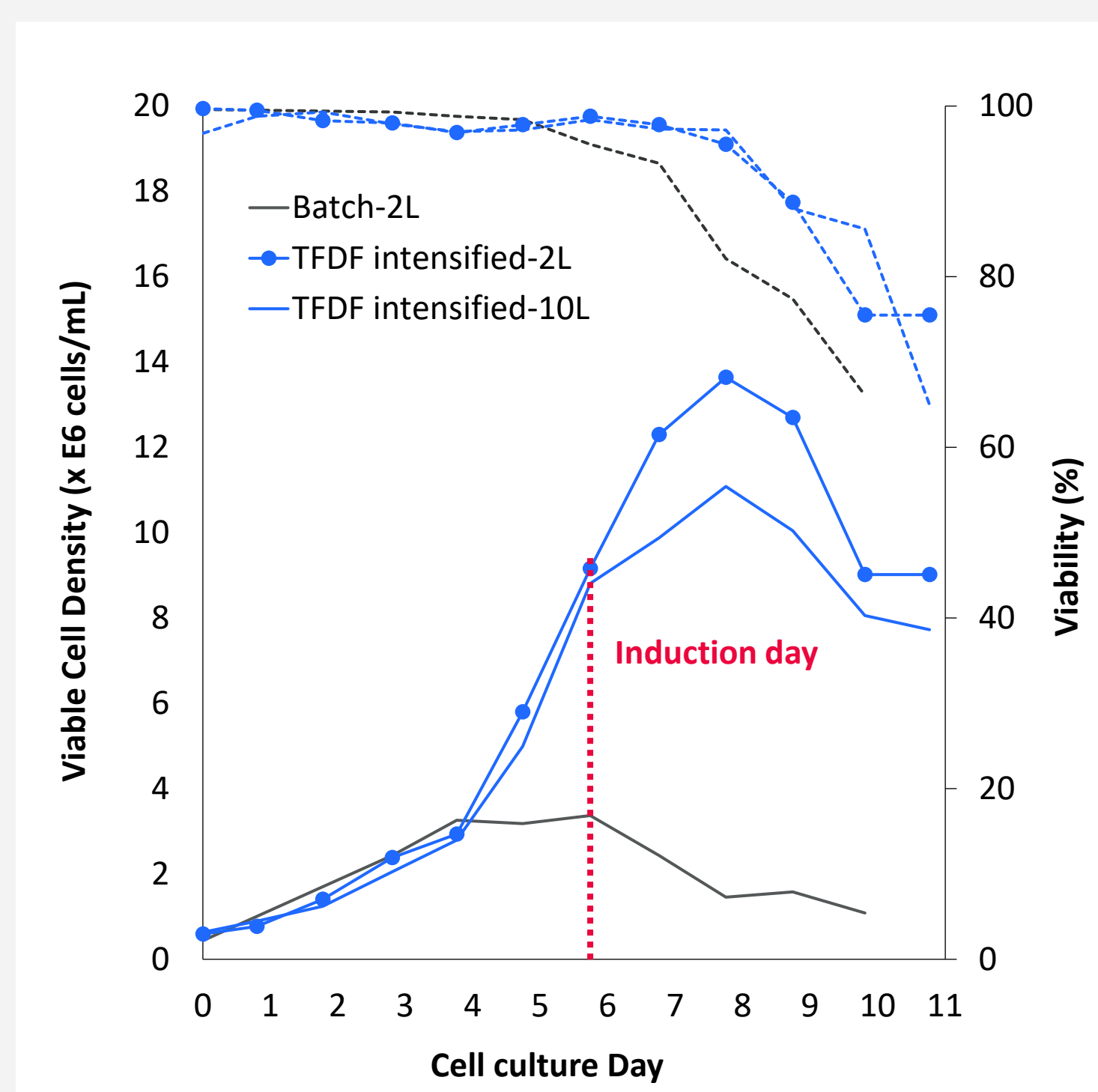
- Cell Line & Culture Conditions:** HEK-based LV producer cells were cultured under stable conditions (≥95% viability, ≤30h doubling time) using at 37°C, 8% CO₂, and 125 rpm.
- Bioreactor Setup:** BioBLU® 3c and 10c vessels with BioFlo® 320 controllers were used, maintaining consistent agitation, temperature, pH, and gas flow rates across 2L and 10L scales.
- TFDF Perfusion Process:** TFDF-based perfusion bioreactors (2L and 10L) were operated using Repligen's KrosFlo® systems, with perfusion rates adjusted based on cell density (1–2 VVD).
- Induction for LV Production:** Lentiviral production was induced using coumestrol and cumate, with sodium butyrate added post-induction to enhance yield; concentrations were maintained throughout production.
- Sampling & Monitoring:** Daily samples were collected post-induction for 5–7 days from bioreactor and permeate lines, analyzed for cell density, viability, metabolites, and LV harvest.
- Titer Assay:** LV titers were measured using a cell-based potency assay involving HEK 293A cells, spin-inoculation, FACS analysis, and GFP gating to calculate transducing units (TU/mL).
- Post-TFDF filters were purchased from several suppliers.**
- Filtration tests using the KrosFlo® KR2i TFF system:**
 - Buffer flush (50 L/m²), then viral load application at 115 LMH.
 - Turbidity, filter feed pressure and permeate volume monitoring until max feed pressure of 20 psi or 1 L filtrate reached.
 - Post-filtration buffer flush with 10-15 mL at 115 LMH.
 - Filtrate pool frozen for later analysis.

Feedstock	Primary Clarification		Secondary Clarification	
	Filters	Pore size range (µm)	Customer filters	Nominal pore size range (µm)
TFDF permeate	TFDF	2 – 5	B (Synthetic polyacrylic fibers)	0.5 – 9
			C (Polyethersulfone)	0.2
			D (Cellulose, inorganic filter aids)	0.4 – 0.8

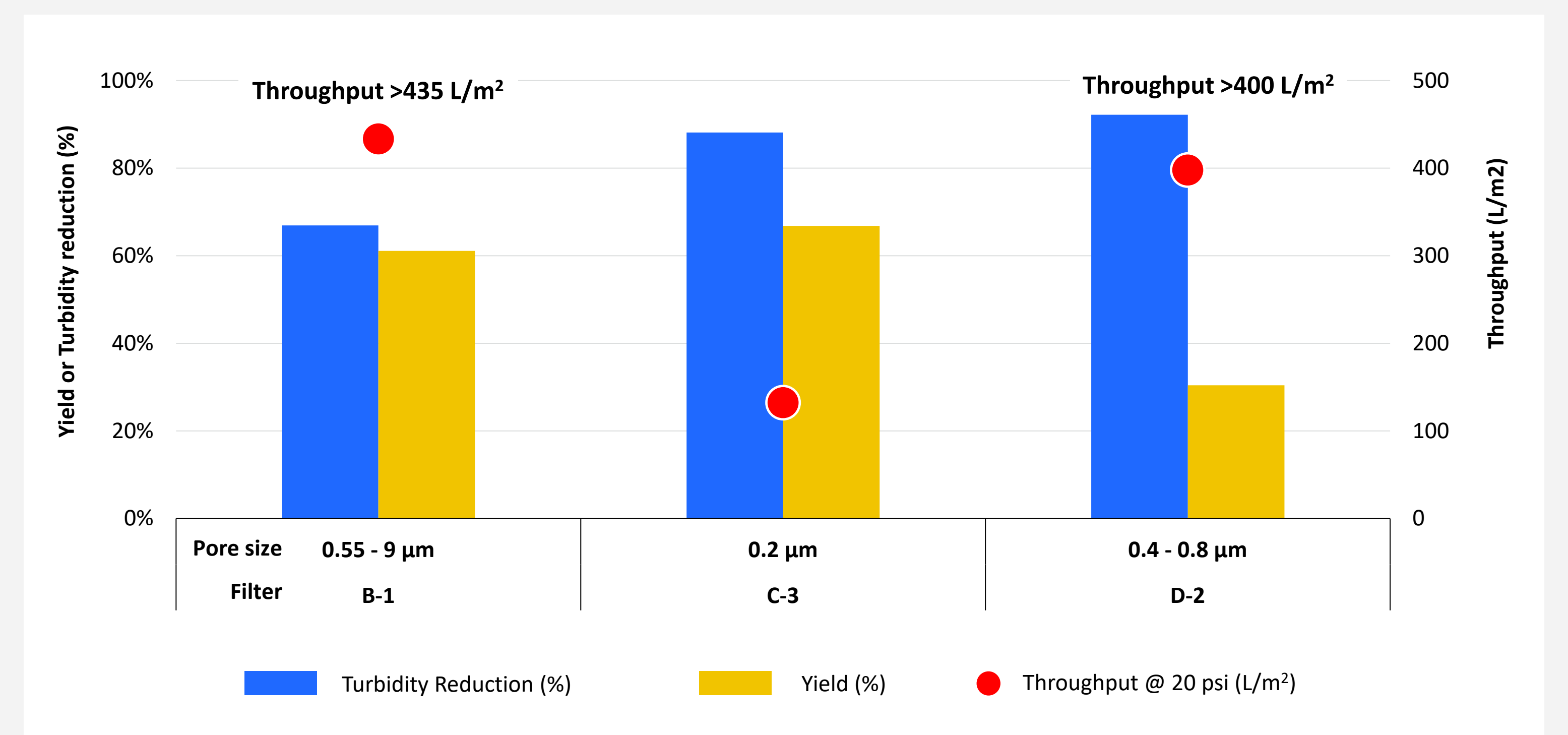


KrosFlo® KR2i TFF System

Batch vs TFDF-intensified LV production



LV TFDF permeate clarification

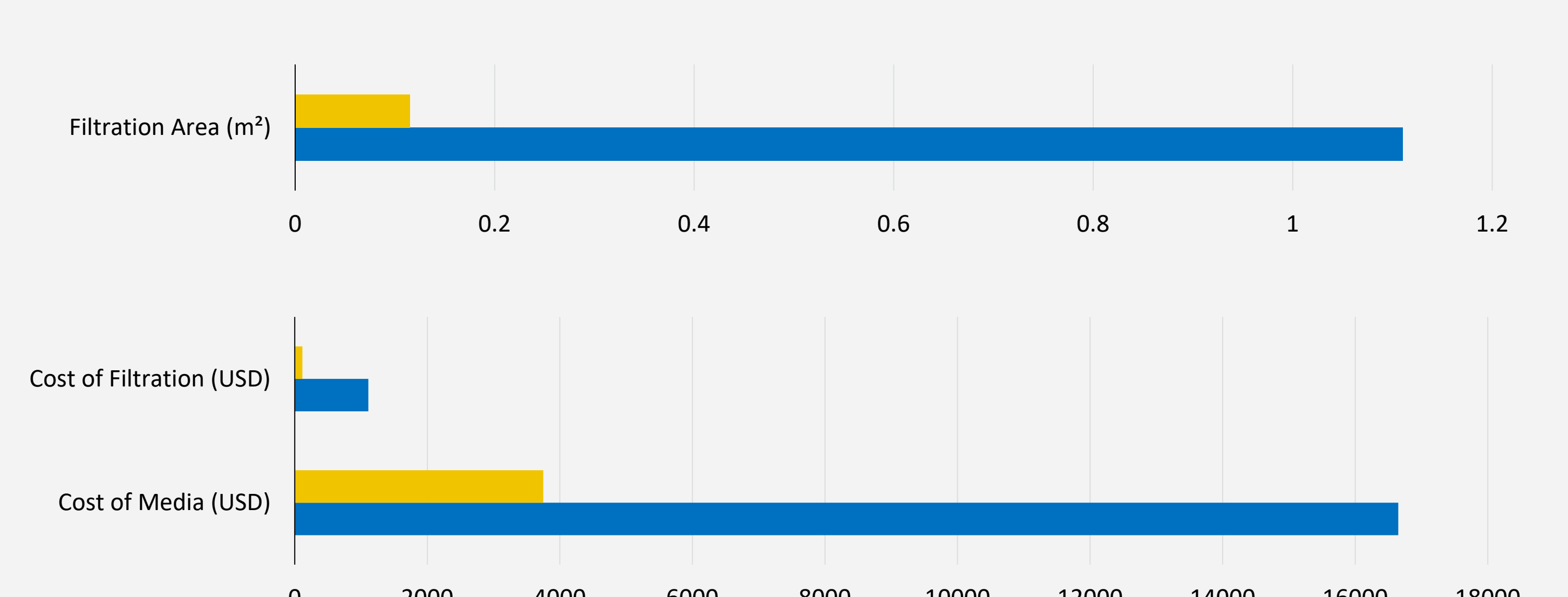
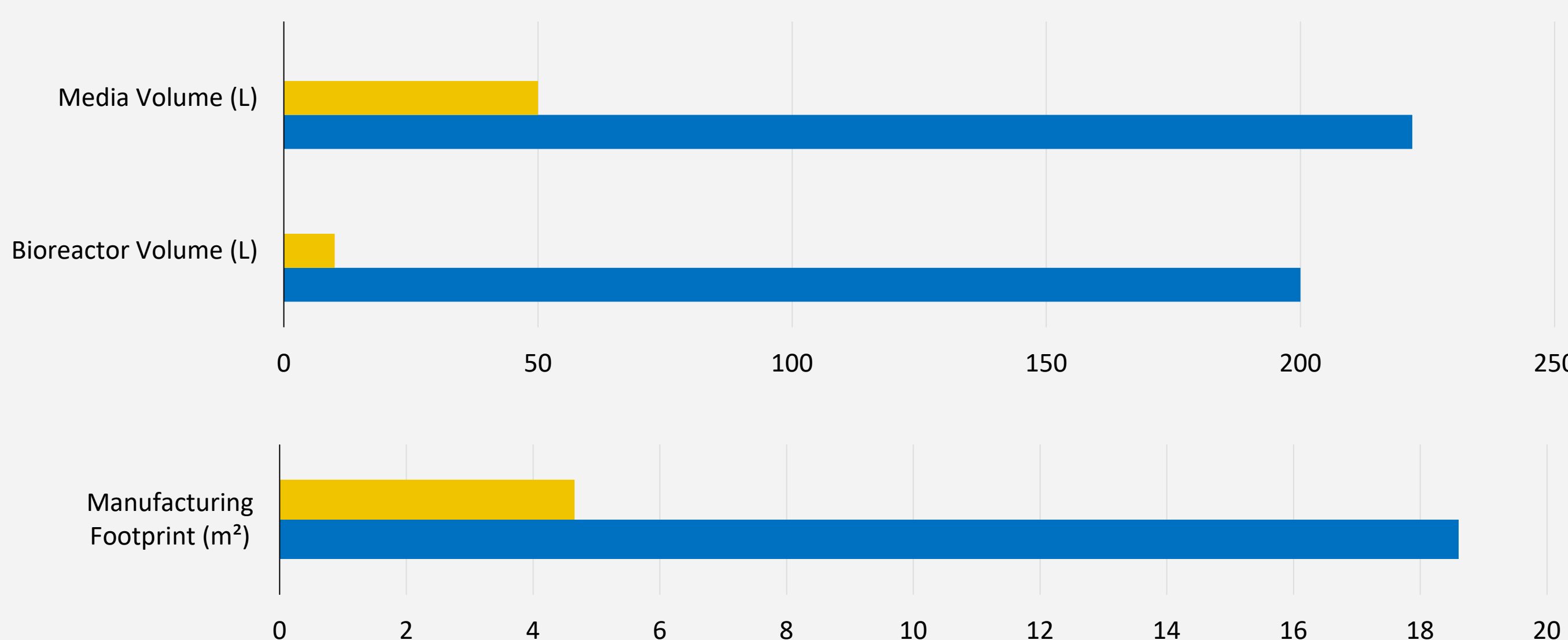


Facility fit for CAR-T clinical manufacturing scenario

CAR-T clinical trial scenario overview Target: 100 patients; Dose Requirement: 1×10^9 TU/patient; **Total LV Needed:** 5×10^{11} TU @ 20% overall process recovery yield

Process assumptions: \$75/L media and \$1000/m² filtration; 200 and 435 L/m² clarification throughput respectively for batch and TFDF-intensified processes

Manufacturing footprint estimate considering space occupied by the bioreactor system and associated upstream/downstream equipment



Conclusion

TFDF® intensification boosts LV yield >10X and enables Phase II/III CAR-T trial production at 10 L scale.

Clarification is streamlined with >2X filtration throughput, >5X reduction in filtration area, and lower turbidity (<60 NTU), reducing cost and footprint significantly compared to 200 L batch processes.

TFDF technology thus enhances both productivity and process simplicity, accelerating the accessibility of CAR-T and other gene therapies.