

High cell density cell cultures (>100E6 c·mL⁻¹) in 2D bags with integrated filter for seed train process intensification

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Introduction

Robust and efficient seed trains are essential for reliable bio-manufacturing processes. Historically certain seed ratios [1] (e.g. 1:2 - 1:10) have been used to inoculate the next bioreactor. Applying perfusion technologies yielding high cell densities during seed trains, a) allows for reducing the number of cultivation steps, thus minimizing seed train equipment and effort, and b) allows for the transfer of high cell densities into the subsequent step, thus supporting e.g. high cell density (HCD) cell banking or HCD inoculation of the main (fed-batch) bioreactor. This strategy is acknowledged and promoted by the biopharma industry and the corresponding consortia (e.g. BPOG).

In this study simple to operate 2D bags with integrated perfusion filter are tested for seed train process intensification.

Material & Methods

Media development

The presented perfusion protocol for high cell density seed trains was developed based on a CHO-DG 44-platform fed-batch process (Sartorius Stedim Cellca). The fed-batch platform media comprised a process medium and two feeds (FMA and FMB). By DoE-based blending studies the corresponding ratios were adapted to maintain high cell densities in shake flasks at 1 volume exchange per day.

Biostat® RM cultivations

After successful implementation of the perfusion process in shake flasks the process was transferred to Biostat® RM 2 L-scale (operated at 50 % working volume). The cultures were maintained at 36.8 °C, pH 7.1 and DO 60 %. An oxygenation cascade was implemented to maintain DO by increasing the rocks per minutes at 10° angle. The perfusion rate was adjusted according to viable cell concentration to maintain a cell specific perfusion rate of 50 pL·(c·d)⁻¹.

Subsequent fed-batch cultivations in shake flasks

To prove the suitability of the high cell density seed train cultures for subsequent cultivations, shake flasks were inoculated at d6 and d7 and operated in fed-batch mode using standard conditions. Their performance was compared to empirical growth and viability data from the standard fed-batch platform process in Biostat® STR scales.

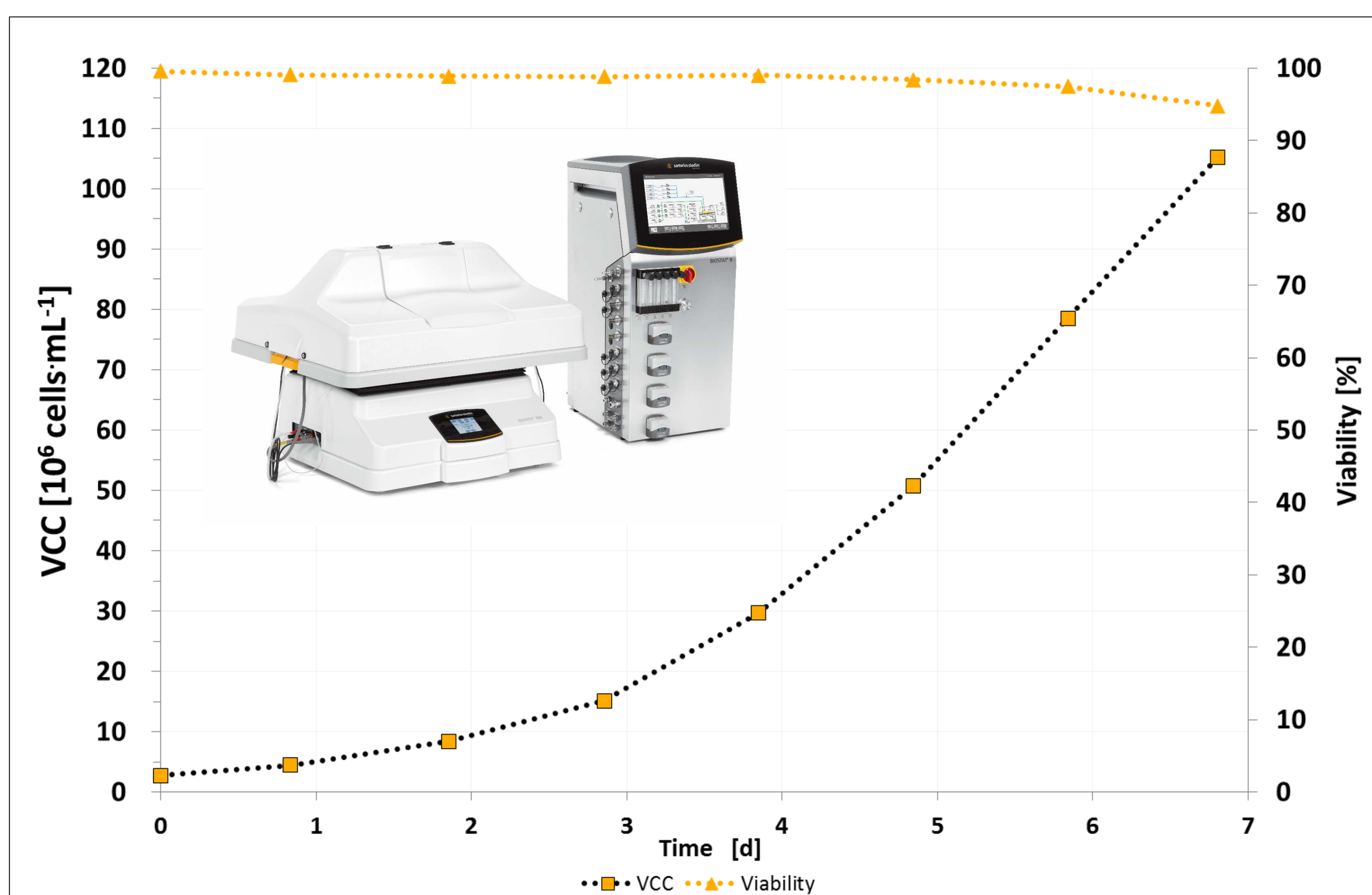


Figure 2: Viable cell concentrations and viabilities of a high cell density cultivation in Biostat® RM systems.

References

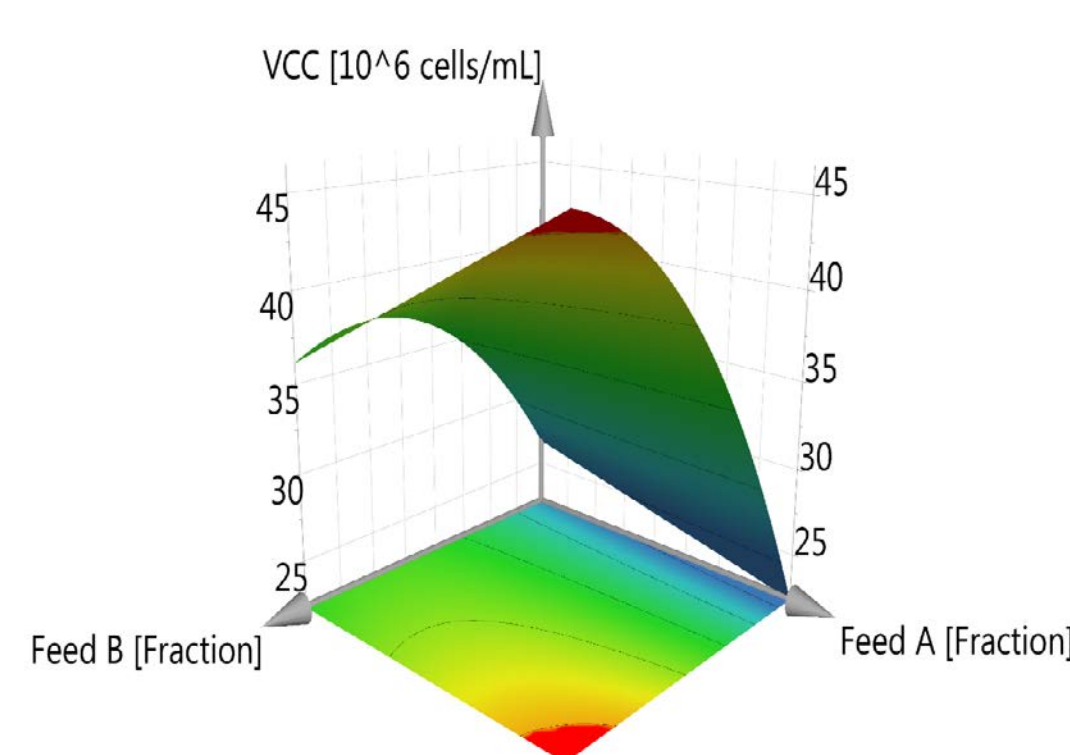
[1] Wittmann, C., Lia, J. C., Lee, S. Y., Nielsen, J., Stephanopoulos, G., Industrial Biotechnology: Products and Processes, Wiley-VCH Verlag GmbH & Co. KgaA, 2017

Acknowledgements:

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CHO-platform fed-batch process (Sartorius Stedim Cellca)

DoE-based blending studies using shake flasks



development of a perfusion process in shake flasks

process transfer to RM-platform

high cell density seed train in Biostat® RM-systems

inoculation of CHO-platform fed-batch process from high cell density seed trains

Fig. 1: Development of a protocol for high cell density seed trains

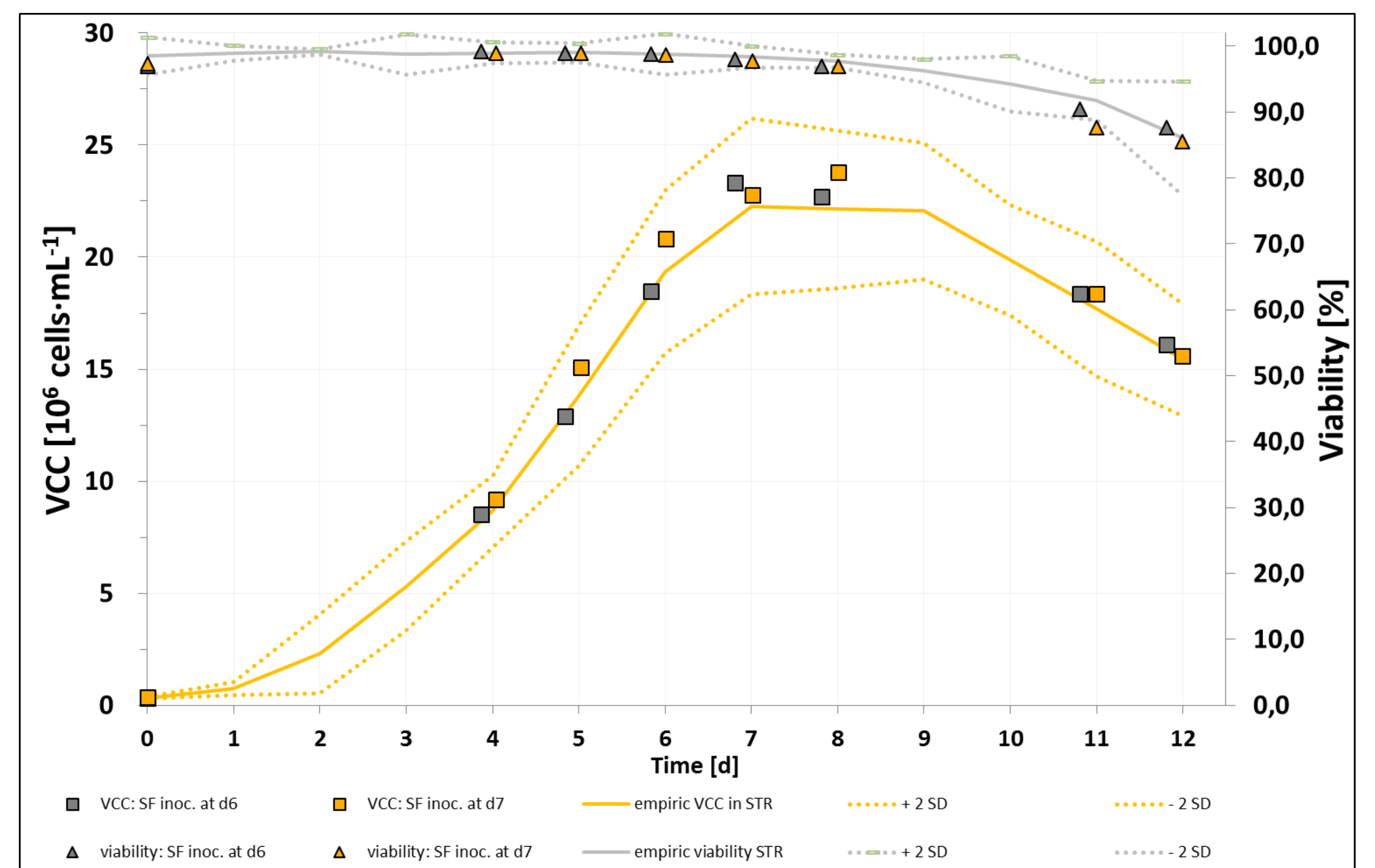


Figure 3: Standard fed-batch processes in shake flasks inoculated from high cell density cultivations in Biostat® RM-systems (Fig. 2). Inoculation took place on d6 and d7.

Results & Discussion

Transferring the newly established perfusion protocol into Biostat® RM-system at 2 L scale resulted in 100E6 c·mL⁻¹ at d7 having viabilities of more than 90 % (Fig. 2). This indicated that the oxygen transfer for the Biostat® RM-system was sufficient to maintain very high cell densities. Fed-batch processes in shake flasks were inoculated with samples taken from the Biostat® RM-system at d6 and d7 (Fig. 3). The processes were very well in the growth trajectory of historic fed-batch processes runs in Biostat® STR-systems. This indicated the general good condition of the seed culture. The overall amount of product expressed in the high cell density seed train was quantified and compared to the productivity in the standard fed-batch process (Fig. 4). The total amount of product produced per liter bioreactor volume increased by a factor of 4 highlighting this protocol's capability for future production.

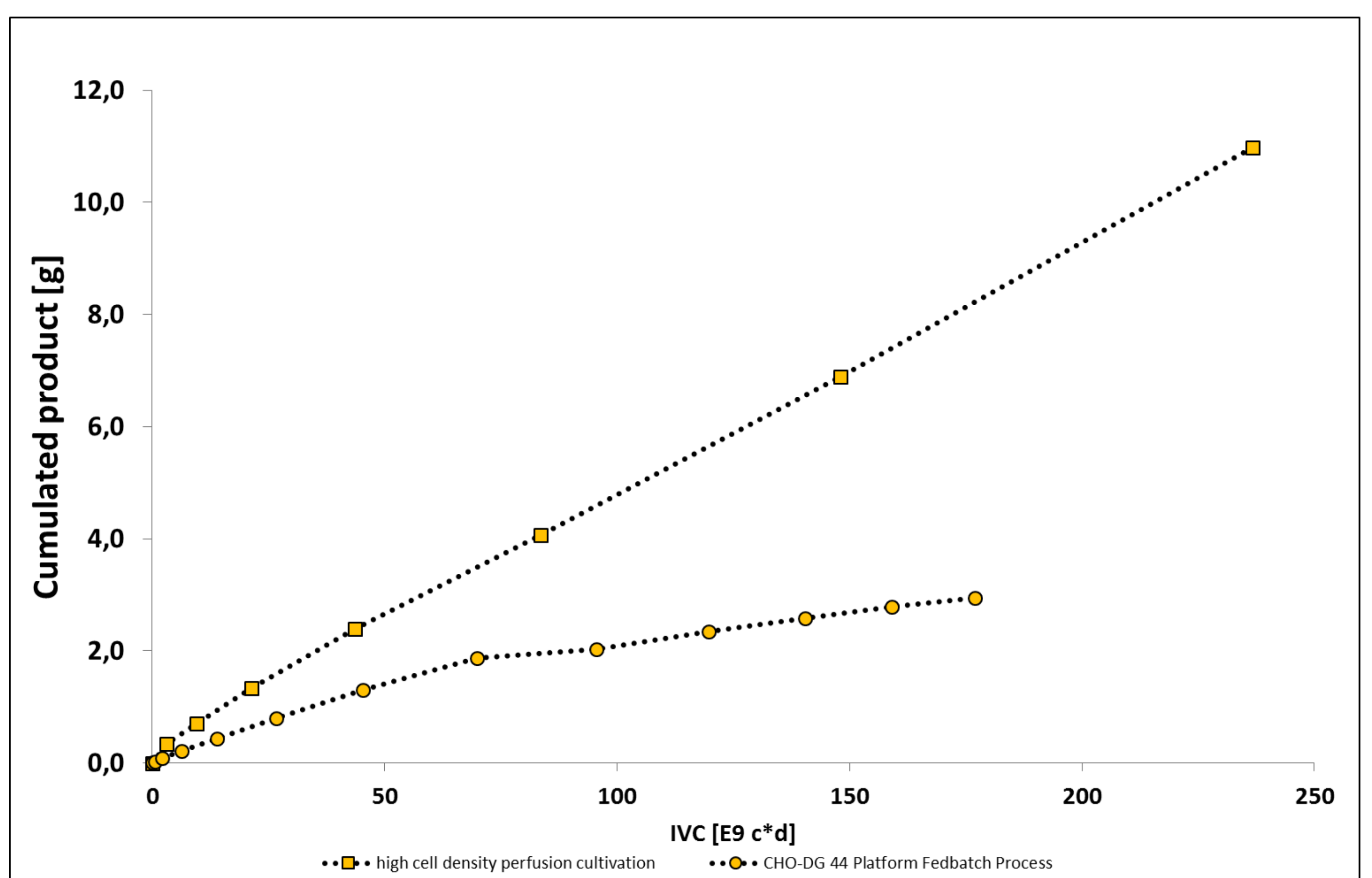


Figure 4: Amount of product produced in high cell density perfusion cultures and standard fed-batch processes depending on the integral of viable cells (IVC).

Conclusions

- The Biostat® RM 2 L is suitable to generate high cell density seed cultures for subsequent processes, e.g. HCD cell banking or subsequent seed train steps.
- The implemented perfusion process increased the production of antibody significantly, the total amount of protein increased by a factor of 4 (in 7 days) compared to the standard (12 day) fed-batch process (normalized to 1 L bioreactor volume).

Outlook

The presented approach demonstrates the suitability of Biostat® RM and Flexsafe® RM with integrated perfusion membrane for high cell density cell culture. Next steps will include implementing the established perfusion protocol in RM systems at larger scale (e.g. to meet requirements for N-2 and N-1 perfusion processes) and automated perfusion operation using the integrated BioPat® ViaMass technology for online biomass monitoring and control.