High cell density cultivation of PER.C6® cells in the CELL-tainer® single use bioreactor

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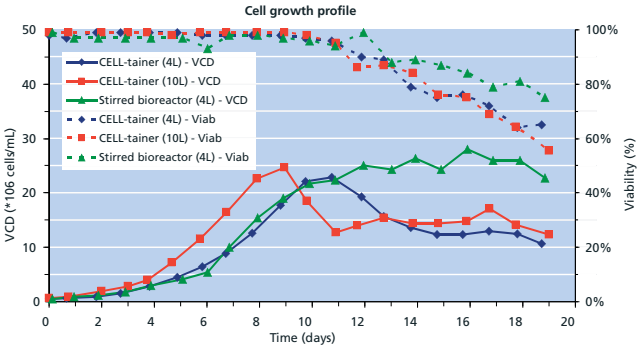
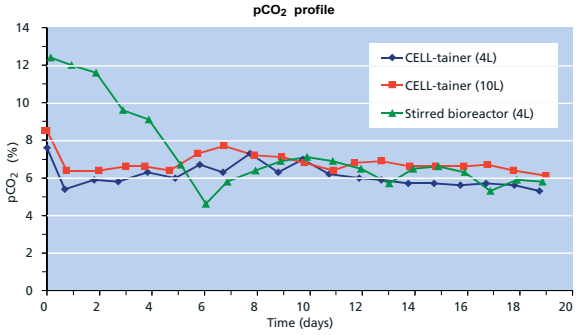
Introduction

A PER.C6® fed-batch platform process is available for the supply of clinical and commercial grade material, that is capable of delivering up to 6-8 g/L of IgG in 250 L stirred bioreactors. In order to implement technologies for straightforward and fast track supply of representative product (10-100 g) for pre-clinical applications and downstream processing development, the PER.C6® fed-batch process was downscaled in rocking-type bioreactors, which are easy to control and require limited operator skills and infrastructure. Because of the high mass transfer rate necessary to support high cell density fed-batch PER.C6® cells, the CELL-tainer® single use bioreactor was selected for this study (offering a $k_La \geq 100\ hr^{-1}$, which is far above the required need for oxygen consumption). The suitability of the CELL-tainer® for the PER.C6® fed-batch process platform was tested using two different working volumes (4 L and 10 L) and the performance was compared, with respect to cell growth and IgG productivity, to a conventional stirred tank bioreactor (4 L working volume).

Methods

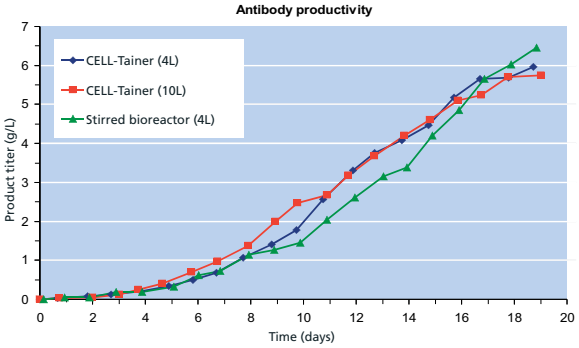
All fed-batch processes were performed in PerMab (HyClone) as basal medium and using the same continuous feeding strategy based on glucose consumption for proper addition of chemically defined feeds and bolus. The stirred tank bioreactor was a 5L Sartorius vessel equipped with a micro-sparger, operated at 0.05 vvm, using a gas mix station with air, N_2 and O_2 to control DO (50%). The DO in the CELL-tainer® was not actively controlled. A flow of air with 5% CO_2 was applied through the headspace (0.3-1.0 L/min), without any oxygen enrichment. Initial rocking speed was 16 rpm for the 4 L process and 18 rpm for the 10 L process, at the low angle setting. Rocking speed and angle were adjusted during the runs according to cell growth and viability. For all the runs, initial pH was 7.3-7.5; pH was not actively controlled. Inoculation cell density was 0.5 $x10^6$ cells/mL for all the experiments.

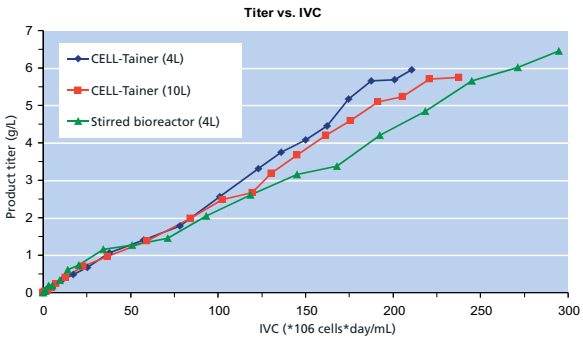




Results

The cell profile comparison shows that PER.C6® cell densities as high as 20-25 $x10^6$ cells/mL can be achieved in the CELL-tainer operated at two different working volumes, resulting in an exponential growth phase very comparable (or even somewhat better; 10 L volume process) to the stirred bioreactor. In contrast cell densities observed during the stationary phase were lower (~15 $x10^6$ cells/mL); further investigation is required for explaining (and potentially circumvent) this different behavior. Beyond generating enough mass transfer for supporting high biomass concentrations, the two-dimensional rocking motion of the CELL-tainer® also provided the stripping capacity required to keep pCO_2 levels below 8-10%. Interestingly, despite the lower cell numbers, the productivity was also similar to that normally observed in stirred bioreactor; IgG titers of around 6 g/L were achieved after 19 days for both the tested volumes. The titer vs. IVC plot shows that specific productivities in the CELL-tainer® were comparable or, at high IVCs, even higher than the conventional bioreactor, indicating that PER.C6 cells are not adversely affected by the different mixing conditions and maintain the ability to produce high titers of IgG antibody during the whole process.





Conclusion

Main conclusion of this study is that the CELL-tainer® is able to support PER.C6® cell densities of over 20 $x10^6$ cells/mL in fed-batch mode, with minimum process control and without the need of a complex infrastructure. Such a process makes it possible to obtain yields of 60-80 g of IgG per batch in a 10 L rocking-type single use bioreactor.

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PER.C6® cells are proprietary technology of DSM/Crucell for the production of monoclonal antibodies and therapeutic proteins. CELL-tainer® is a registered trademark of CELLution Biotech BV.

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