

# Cultivation of PER.C6<sup>®</sup> cells in the novel CELL-tainer<sup>™</sup> high-performance disposable bioreactor

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## Introduction

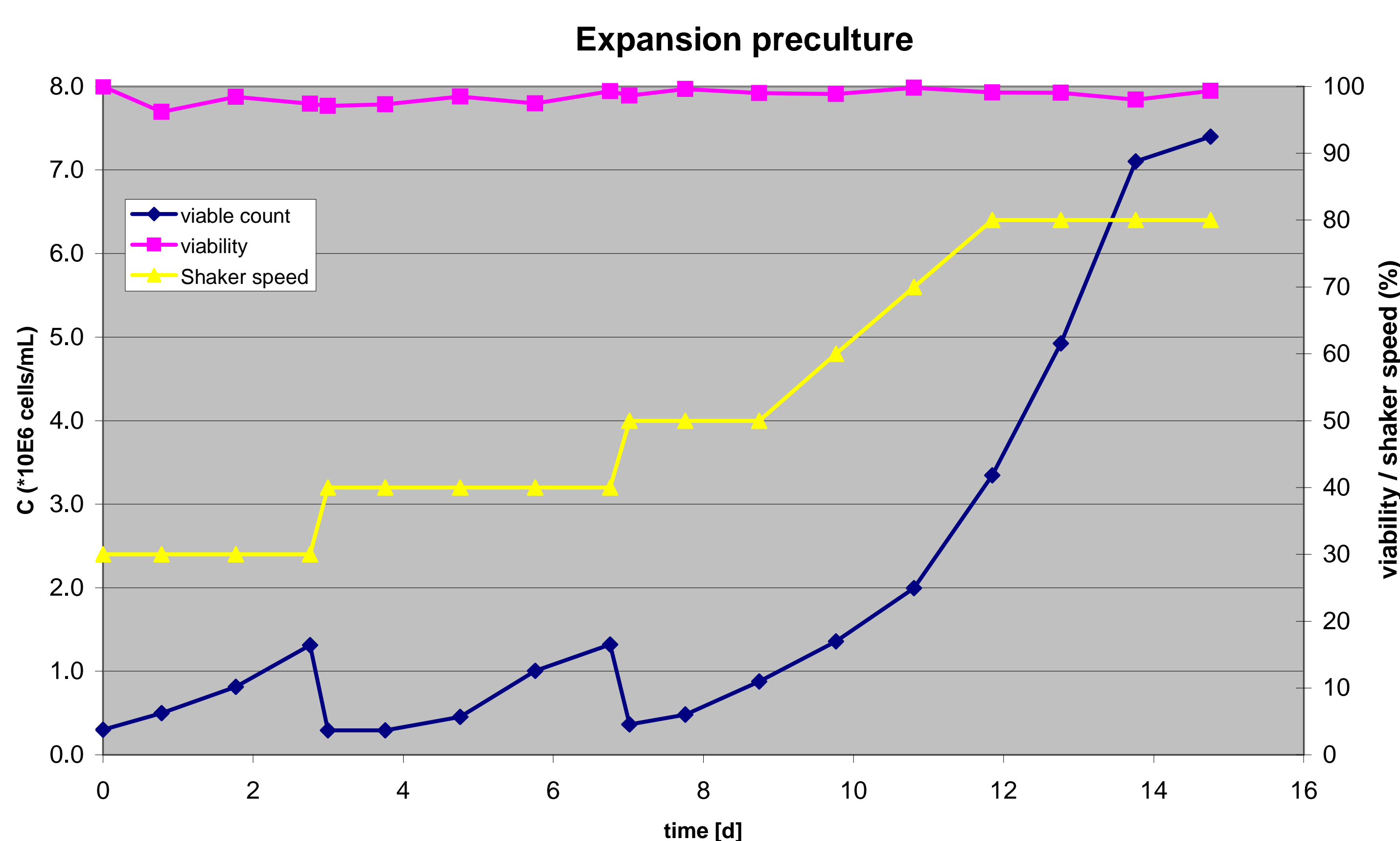
The CELL-tainer<sup>™</sup> is a novel wave-motion bioreactor characterized by very high mass-transfer capacities ( $k_L a$  up to  $200 \text{ h}^{-1}$ ) due to an optimized wave motion. By comparison, this mass-transfer capacity is at least a factor 5 higher than that of stirred tank cell-culture bioreactors. As such, no oxygen supplementation of the overlay is required for oxygenation and the carbon dioxide stripping capacity is sufficient to prevent  $\text{pCO}_2$  build-up.

In this study for the first time animal cells were cultured in the CELL-tainer<sup>™</sup>. First the system settings were optimized for an antibody producing PER.C6<sup>®</sup> clone during a serial expansion culture. Subsequently the growth performance in the CELL-tainer<sup>™</sup> was compared with a conventional wave-motion bioreactor and a stirred tank bioreactor.

## Materials and Methods

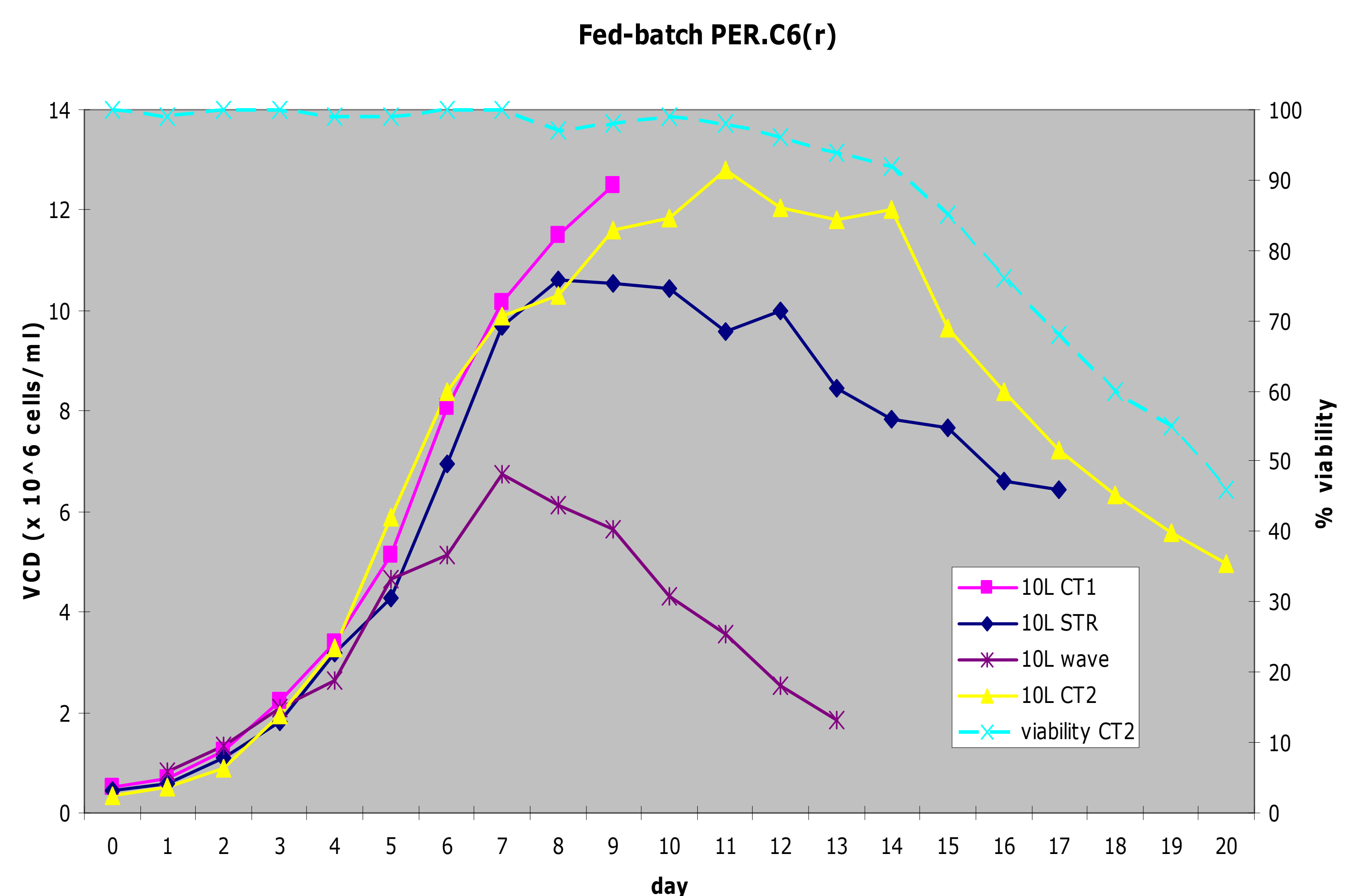
All experiments were performed using VPRO (SAFC) as basal medium. The Fed-batch comparison experiments were performed at 10 L scale using the same bolus feed protocol including glucose, amino acids, trace elements, and Soy-hydrolysate. The same seed culture was used in the comparison experiments.

The bioreactor was a 10 L Sartorius vessel equipped with a ring sparger, operated at 0.1 vvm, using a gas mix station with Air,  $\text{N}_2$ ,  $\text{O}_2$  and  $\text{CO}_2$  to control DO (50%) and pH (6.8 – 7.2). The DO in the conventional wave-motion bioreactor (Wave Biotech) and the (prototype) CELL-tainer<sup>™</sup> were not actively controlled. A headspace flow of air with 5 %  $\text{CO}_2$  was applied. No upward pH control with 0.1 M NaOH was applied unless pH was below 6.8. Rocking speed and angle in the conventional wave-motion bioreactor were 20 rpm and 7°. The CELL-tainer<sup>™</sup> was operated at 17 – 21 rpm at the low angle setting.



## Results and Discussion

Serial passaging in the CELL-tainer shows that the intense mixing in the CELL-tainer<sup>™</sup> does not adversely affect PER.C6<sup>®</sup> cell growth up to 80 % (at the low angle setting). In fed-batch cell densities of over  $12 \times 10^6$  cells/mL were achieved. Manual upward pH control was applied from day 16 onward. Higher cell densities, thus higher product titers may be possible from a mass-transfer perspective. The growth performance was even somewhat better than in a stirred tank bioreactor with full process control. Due to the improved longevity also the productivity in the CELL-tainer<sup>™</sup> was significantly higher than in the conventional wave-motion bioreactor and somewhat higher than in the stirred tank bioreactor.



## Conclusions

The CELL-tainer<sup>™</sup> is able to support commercially relevant PER.C6<sup>®</sup> cell concentrations of more than  $12 \times 10^6$  cells/mL at 10 L scale in Fed Batch mode with minimum process control.

## Acknowledgements

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The PER.C6<sup>®</sup>-celline is proprietary technology of Crucell / DSM for the production of monoclonal antibodies and therapeutic proteins. CELL-tainer<sup>™</sup> is a registered trademark of CELLution Biotech BV.